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THE KAROLINSKA INSTITUTE

DEPARTMENT OF ENVIRONMENTAL HYGIENE

CADMIUM IN THE ENVIRONMENT, II

By

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A report is presented which, as an update of an earlier review, focuses upon information essential to the understanding of the toxic action of cadmium and the relationship between exposure and effects on human beings and animals. The various chapters review information about the following aspects of cadmium: (1) analytical methods of measuring cadmium; (2) occurrence, possible routes of exposure and daily intake; (3) metabolism, including the following: uptake and absorption, transport, distribution and excretion of cadmium in animals, and in normal and exposed human beings; (4) relationships among concentrations of cadmium in blood, urine and organs, Metallothionein; (5) effects and dose-response relationships on bone, and liver, renal effects; (6) epidemiological investigations in cadmium-polluted areas of Japan discusses methodology and cadmium exposure and medical effects in individual areas; (7) the cause of the Itai-Itai disease.

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CHAPTER 1 INTRODUCTION

This report updates the earlier review on cadmium carried out under a contract (No. CPA 70-30) between the US Environmental Protection Agency and the Department of Environmental Hygiene of the Karolinska Institute, Sweden. The earlier report, with modifications, has also been published as a monograph, Friberg, L., Piscator, M., and Nordberg, G.: Cadmium in the Environment, CRC Press, Cleveland, 1971 (in the following referred to as CITE). The present report is a result of a continued collaboration (contract No. 68-02-0342) between the two institutions and covers available information, published as well as unpublished, up to and including 1972. The project officer of the US Environmental Protection Agency for this project has been Robert J.M. Horton, M.D.

Like the previous review, the present work focuses upon information essential to the understanding of the toxic action of cadmium and the relationship between exposure and effects on human beings and animals.

Through repeated personal contact with several Japanese researchers, including a five-week visit there by one of us (Tord Kjellström), it has been possible to obtain and evaluate much data from Japan which would not be available otherwise. Papers published in Japanese could be taken into account as well since one of us (Tord Kjellström) speaks and reads Japanese.

We express our gratitude to the Environmental Agency of Japan, particularly Dr. Yoshimasa Yamamoto, Chief of the Section of Environmental Health and Public Hazards, as well as to the Prefectural Institutes of Hygiene and Departments of Public

Hazards in Fukushima, Gumma, Hyogo, Nagasaki, and Toyama, as well as the Japanese Association of Public Health. Valuable information and assistance have been received from several independent researchers. Kenzaburo Tsuchiya, M.D. Professor of Preventive Medicine and Public Health, School of Medicine, Keio University, assisted us in planning and executing valuable visits in Japan.

We express our thanks to Ms. Pamela Boston, B.A., for assistance in editing the English of the report.

CHAPTER 2 ANALYTICAL METHODS

In our previous review Cadmium in the Environment (CITE) it was concluded that there are methods which are reliable for the determination of small amounts of cadmium in biological material. At the same time, it was warned that several erroneous results have been reported in the past because of interferences, most noticeably the interference of salts, especially sodium chloride, in atomic absorption spectrophotometry analyses which failed to take these into account.

During the last years, further experience has resulted in a large number of articles on methods of analysis of cadmium. Yet, systematic studies on accuracy, precision and other important parameters have seldom been performed. In addition to dithizone methods, emission spectroscopy, neutron activation and atomic absorption spectrophotometry, which were treated in CITE, some new developments are flameless atomic absorption, electrometric methods (anodic stripping polarography, pulse polarography, anodic stripping voltammetry). The newly developed X-ray analysis after proton irradiation is also of interest. Information about analytical methods can also be found in a recent monograph, Cadmium, the Dissipated Element, by Fulkerson et al., 1973.

2.1 ATOMIC ABSORPTION

For the analysis of cadmium in whole blood, Hauser, Hinners and Kent, 1972, have developed a technique whereby 0.5 ml blood is added to a flame-purged tantalum sampling boat, dried in an oven and ashed at low temperature. Analyses is then performed by flaming the ash directly in an atomic absorption spectrophotometer with a deutrium background corrector. The method was used for twelve replicate analyses of a blood sample and gave an average of 0.35 µg Cd/100 ml blood with a variation coefficient of 27%. Recovery, estimated by adding 10 µg Cd/ml blood to another sample, was reported to have been complete. The detection limit is given as 0.02 μg Cd/100 ml blood, based on a pure water standard solution of cadmium. The detection limit of cadmium in blood is not known.

Lener and Bibr, 1972, made some methodological studies on atomic absorption spectrophotometry. They used an air propane flame instead of the conventional air acetylene flame and state that by using the former system, cadmium can be analyzed in concentrations from 0.06 ppm. The authors found that the coefficient of variation was 3.8% at a concentration of 0.06 ppm. This was based on 20 repeated measurements of cadmium in water solution. When applied to food, 4 analyses of the same sample of parsley gave the result of 0.088 ppm with a standard deviation of 0.077. (In the article, the term "standard error" is used, but must mean standard deviation). For a sample of carrot the coefficient of variation for 5 analyses was about 30%.

For determination of cadmium in urine and blood, Vens and Lauwerys, 1972, passed urine acidified with 1.5 N HCl or hemolyzed blood in 1.5 N HCl through a basic resin, which binds cadmium. Elution was performed with 2 N perchloric acid and cadmium was then determined with conventional atomic absorption spectrophotometry. The coefficient of variation was 10% at a cadmium concentration of 1.5 μg/l. The accuracy is not known. In 44 normal subjects the mean urinary concentration was 1.37 μg/l (S.D. 1.38) or 0.95 μg/g creatinine (S.D. 0.72). In 24 normal subjects the mean concentration in blood was 0.95 μg/100 ml (S.D. 1.0), which is higher than earlier reported.

In CITE it was mentioned that extraction methods followed by atomic absorption analysis were reliable for the determination of cadmium in urine. It was also stated that good agreement was obtained in Japan among different laboratories for the same urines. Similar interlaboratory studies have been performed in Japan on rice samples (Yamagata et al., 1971). Ten laboratories participated using a standard method of analysis for determination of cadmium in rice. Some of the steps are wet ashing with nitric acid and sulfuric acid, extraction with APDC-MIBK and measurement by atomic absorption. The means for two rice samples were 0.176 and 2.33 ppm and the ranges 0.14-0.20 and 2.20-2.75 ppm, respectively., giving a coefficient of variation of about 10 percent for both samples. Since it is difficult to obtain homogenous rice samples, these results indicate a high degree of accuracy in these Japanese laboratories. In one of the laboratories, a comparison was made between a method involving low temperature ashing followed by extraction into acid and the above mentioned standard method (chloroform used instead of MIBK). Eight determinations on the same rice sample gave 0.188 (S.D. 0.005) and 0.189 (S.D. 0.014) ppm respectively.

The above mentioned results indicate that the Japanese data for cadmium in rice can be regarded as generally very reliable, which is of importance when discussing exposure (see Chapter 6).

For determination of cadmium in grains a method using flameless atomic absorption has been developed by Kjellström et al. (to be published). Radioactive ^{109}Cd was added to 2-gram and 4-gram samples of wheat. Radioactive analysis was compared with flameless atomic absorption and conventional atomic absorption (with and without extraction in MIBK/APDC system). The deutrium lamp technique was used for background correction. The flameless atomic absorption was performed in the following way. The sample was dry ashed twice and the ash was dissolved in 1-M HNO_3 and 20 µl of this solution was injected into a Perkin-Elmer HGA-70 heated graphite atomizer. This method showed a higher repeatability than conventional atomic absorption and the recovery (average 95%) was about 10% higher than for the method using extraction. In Figure 2:1 is shown the result of flameless atomic absorption analysis of cadmium. The accuracy of the new method for cadmium analysis was studied by Linnman et al. (to be published), who compared it with neutron activation (method of Ljunggren et al., 1971). The results are shown in Figure 2:2. A good correlation between the methods is shown at cadmium concentrations in wheat between 20 and 200 ng/g.

2.2 ELECTROMETRIC METHODS

Basically related to the classic polarographic method, these methods have been improved to increase their sensitivity. Anodic stripping voltammetry (ASV), which utilizes either mercury electrodes or solid electrodes, has been used in several investigations for the determination of cadmium and other metals in water (Whitnack and Sasselli, 1969; Allen, Matson and Mancy, 1970), in food (Hundley and Warren, 1970) and in blood serum (Sinko and Gomiscek).

The ASV-method was compared with another method only in the report involving the food samples (Hundley and Warren, 1970). Here it was compared with atomic absorption spectrophotometry, but it was not stated how this latter method was performed. There seems to have been a poor agreement between the two methods and these data do not not help to assess the value of the ASV-method.

Pulse polarography was used by Abdullah and Royle, 1972, to determine cadmium in sea water after pre-concentration of metals on resins. Only one value is given for cadmium in sea water, 1.38 µg/l and this method cannot be evaluated at present.

2.3 X-RAY ANALYSIS

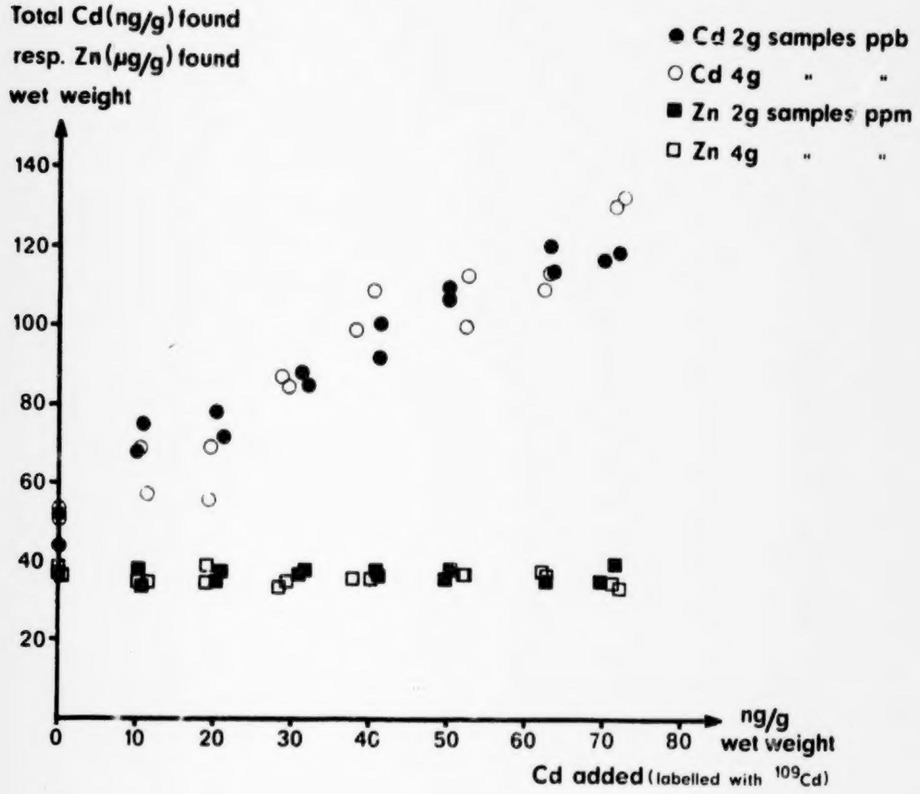
Johansson, Akselsson and Johansson, 1970, showed that it is possible to detect small amounts of elements by observation of characteristic X-rays aftor proton bombardment. Since this method is non-destructive, repeated analysis can be made on the same sample. As yet, the problem of quantitative determination of small amounts of cadmium in biological fluids has not been solved, but in the future, this method may be of great value for cadmium analysis.

2.4 CONCLUSIONS

A number of potentially very useful methods for analyses of even very low concentrations of cadmium in biological material are now available. To date, few strict methodological studies on accuracy and other important parameters have been carried out. This is a severe limitation, as witnessed by some recent reports on occurrence and metabolism of cadmium in which serious analytical errors must have been involved. It is therefore necessary to evaluate the analytical methods used before accepting data.

TABLE 2:1 Cadmium content in some foods (µg/g wet weight). (From Lener and Bibr, 1972).

Sample	No. of Analyses	Direct measurement		Measurement after extraction	
		M	S.E.	M	S.E.
Celery	5	0.058	0.013	0.059	0.011
Parsley	4	0.088	0.077	0.086	0.006
Garlic	5	0.077	0.001	0.075	0.001
Carrot	5	0.086	0.024	0.089	0.016
Onion	5	0.047	0.003	0.046	0.002
Potatoes	5	0.092	0.002	0.090	0.002
Milk	5	0.010	0.003	0.012	0.003
Egg yolk	4	0.120	0.005	0.121	0.004
Egg white	4	0.076	0.004	0.073	0.005



Zinc was analyzed with conventional atomic absorption (AAS-F) in order to show that all samples were identical in respect to original metal content.

FIGURE 2:1 Result of flameless atomic absorption cadmium analysis of two different sizes of wheat samples to which cadmium (labelled with ^{109}Cd) had been added. (From Kjellström et al., to be published).

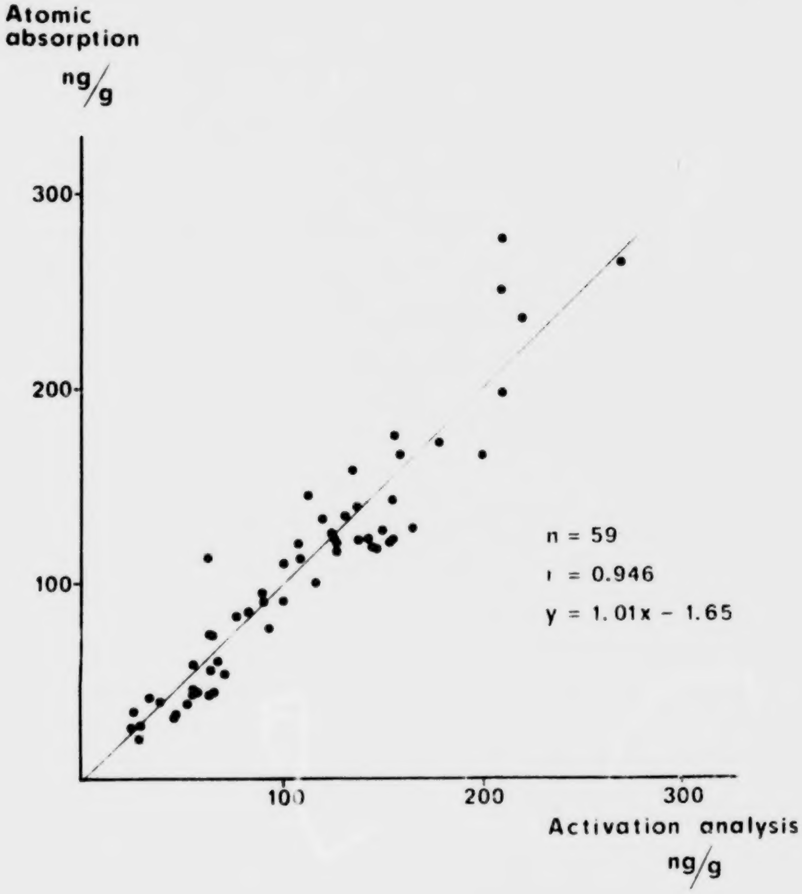


FIGURE 2:2 Result of analysis of cadmium in wheat using flameless stomic absorption method and neutron activation method. (From Linnman et al., to be published).

CHAPTER 3 OCCURRENCE, POSSIBLE ROUTES OF EXPOSURE AND DAILY INTAKE

3.1 OCCURRENCE AND POSSIBLE ROUTES OF EXPOSURE

3.1.1 Cadmium in air

In the previous review (CITE) it was concluded that "normal" concentrations of cadmium in ambient air are 0.001 $\mu g/m^3$ or less in rural areas. In cities mean yearly concentrations of up to 0.05 $\mu g/m^3$ may be found, and in areas near cadmium emitting industries weekly means of 0.3 $\mu g/m^3$ have been recorded.

The values reported have been verified during the last years. Just and Kelus, 1971, found mean yearly concentrations of cadmium in air from 0.002-0.05 $\mu g/m^3$ in 10 Polish towns. Creason et al., 1972, found at 4 sites in Cincinnati, Ohio, cadmium concentrations in air from 0.0001-0.04 $\mu g/m^3$ (means for 3-5 days). The concentrations were about the same at 25 and 100 feet from the roadway indicating that traffic did not influence cadmium concentrations in air.

In the Chicago area Harrison and Winchester, 1971, during 8 24-hour sampling periods from May to August at 50 sampling stations recorded cadmium concentrations in air from less than 0.005 $\mu g/m^3$ to 0.08 $\mu g/m^3$. Severs and Chambers, 1972, found 24-hour values during one day of up to 0.33 $\mu g/m^3$ in Houston, Texas. In the city of East Helena, Montana, the average concentration during a three-month sampling period was 0.06 and 0.29 $\mu g/m^3$ at about 1300 and 800 meters respectively from a smelter (Huey, 1972). The maximum 24-hour value was 0.7 $\mu g/m^3$ (U.S. Environmental Protection Agency, 1972).

gata et al., 1972, report on cadmium concentrations in ambient air in Tokyo during 1969 to 1971. Mean values over several months in different areas varied from 0.010 to 0.053 with a maximum 24-hour value of 0.53 $\mu g/m^3$.

The deposition of airborne cadmium has been further studied in Scandinavia (at about 100 different places) by analyses of cadmium concentrations in moss. As seen in Figure 3:1 there is an obvious difference between the southern and the northern parts of Sweden, Norway and Finland (Rühling and Tyler, 1972). Moss analysis has also been used in Great Britain by Goodman and Roberts, 1971, and Burkitt, Lester and Nickless, 1972, to study the distribution of cadmium pollution. The latter authors found about 50 ppm of cadmium in moss at distances of 6 miles from a smelter. This confirms that considerable air pollution of cadmium may spread to relatively long distances from emission sources.

Secular trends in cadmium deposition have been studied by comparing cadmium concentration measured in moss saved from several decades ago with that in moss from present times. The old samples consisted of moss from the years 1927-1942 (herbaria samples) from 10 sites in the rural areas around the city of Uppsala, Sweden, and moss from 3 sampling sites in the city of Uppsala, collected in 1916-1917.

It was found that in the rural areas around Uppsala there had been a mean increase from 0.2 to 0.3 ppm in moss during the intervening 20-30 years. In the city a mean increase from 0.2 to 0.4 ppm was observed during a 50-year period (Gelting and Pontén, 1971). The increase was consistent in several comparisons.

In a rural, subarctic area in Finland, Laamanen, 1972, found a deposition of cadmium from less than 0.001 mg/m^2/month (winter) to 0.006 mg/m^2/month (summer) by measuring cadmium in dust fall. These data may be compared with similar studies in the city of East Helena in Montana, where values of 1-4 mg/m^2/month were found up to about 1,000 meters from a cadmium emitting smelter (Huey, 1972). These latter data are comparable to those presented in CITE for an area near a Swedish industry (0.4-40 mg/m^2/month).

The relationship between dust fall of cadmium and concentrations of cadmium in air has been studied by Pinkerton et al., 1972.

They found that dust fall values of 0.055 and 0.091 mg/m^2/month corresponded to concentrations of cadmium in the air of 0.0032 µg/m^3 and 0.0048 µg/m^3 respectively. It was also found that in an area with a monthly dust fall of cadmium from 0 to 0.12 mg/m^2/month household dust contained between 4.25 and 12.5 µg Cd/g dust. Corresponding values in an area where the dust fall was from 0.04 to 0.22 mg/m^2/month were 8.75 to 14 µg/g dust.

3.1.2 Cadmium in water

In the previous review it was concluded that in areas not known to be polluted by cadmium, the concentrations of cadmium in water generally are less than 1 ppb, both in natural waters and in drinking water.

In two recent papers from Great Britain, cadmium has been determined in sea water, both on and off coast. Preston et al., 1972, found a geometric mean of 0.04 ppb in 21 samples of filtrated sea water collected in the Irish Sea, whereas near the coast, in the Liverpool Bay, in 9 samples, a geometric mean of 0.41 ppb water was obtained. This latter value is in good agreement with data by Abdullah, Royle and Morris, 1972, who found a mean value in the Liverpool Bay of 0.27 ppb. According to Preston et al., 1972, the highest concentrations in the coastal waters around Britain were found in the North Sea, where the mean was 0.41 ppb.

In drinking water, a WHO working group (World Health Organization, 1972) recommended that cadmium concentrations should not exceed 5 ppb.

3.1.3 Cadmium in soil

In CITE it was concluded that in non-polluted areas cadmium concentrations in soil generally are below 1 ppm, whereas in areas near cadmium emitting industries, considerably higher values may be found. It was stated that information is lacking on what factors determine the uptake of cadmium by plants, and on the extent to which selective mechanisms operate. It was also pointed out that the use of sewage sludges as fertilizers might increase cadmium concentrations in soil.

Odén, Berggren and Engvall, 1970, determined cadmium in sewage sludge from 56 plants in the southern and middle parts of Sweden. The median concentration of cadmium was 12 ppm dry weight (range: 2-61 ppm). Berrow and Webber, 1972, have reported on metals in sewage sludge from 42 sewage works, scattered throughout England and Wales. The samples were collected in 1964. Of these 42 samples, 7 contained more than 100 ppm dry weight.

The cadmium uptake in radish and lettuce was studied by John, Van Laerhoven and Chuah, 1972. Cadmium chloride was added to thirty different soil samples in pots (original cadmium concentrations not given) to give a concentration of 100 ppm, and corresponding samples without added cadmium were used as controls. After a growing period of 3 weeks, a reduced yield of both radish and lettuce was found. Cadmium was determined by atomic absorption with correction for background absorption and light scattering. Cadmium concentrations on a dry weight basis in radish roots were on an average 387 and 7.4 ppm respectively, and in lettuce tops 138 and 2.3 ppm respectively.

The concentrations reported in the control plants are high. There is no doubt, however, that cadmium concentrations in these two foodstuffs greatly increased when grown on cadmium contaminated soil. It was also shown that the uptake was related to pH, i.e. increased soil acidity resulted in higher cadmium concentrations in plants.

Linnman et al. (to be published) studied the uptake of cadmium in wheat grown in test pots at different sewage sludge additions (corresponding to 0 up to 175 tons/hectare) and different pH levels. All samples were analyzed for cadmium both by means of flameless atomic absorption with background correction and by neutron activation (for a comparison of the methods see Chapter 2, Figure 2:2).

The results of the studies can be seen in Table 3:1 where cadmium uptake in wheat in relation to added sewage sludge, cadmium concentration in soil and pH of soil is given. It is seen that a considerable cadmium uptake occurs in wheat when sewage sludge is used as a plant nutrient source. The lower the pH the greater was the cadmium concentration in wheat. It should be mentioned that the two lower levels of added sludge, 6.5 and 19 ton/hectare are in agreement with Swedish recommendations for annual applications (Linnman et al., to be published). These results are from laboratory studies and field studies are now needed.

3.1.4 Cadmium in food

In CITE it was concluded that most foodstuffs contain less than 0.05 ppm cadmium wet weight. Liver, kidney and some marine organisms, such as shellfish, may contain much higher quantities of cadmium, even in non-polluted areas. Rice and wheat, two important foodstuffs, may accumulate cadmium to concentrations of more than 1 ppm in polluted areas. Considerable difficulties in the analysis of the often very low concentrations of cadmium in foodstuffs were acknowledged and some results were regarded as unreliable.

Corneliussen, 1970, and Corneliussen (in press) report on an extensive study of cadmium content in different types of food. The samples were collected from 1968 to 1970, from 30 markets in 24 different cities in the United States. Cadmium was analyzed by atomic absorption and/or polarography at a sensitivity of 0.01 ppm. The results have been compiled in Table 3:2. In most food categories the mean cadmium concentration was less than 0.05 ppm and the maximum concentration below 0.1 ppm.

Zook, Greene and Morris, 1970, determined cadmium in different kinds of wheat, commercially prepared flours from these wheats and products prepared from the flours. With the method used (atomic absorption with background correction) mean cadmium concentrations in wheat varied from 0.07 to 0.13 ppm (dry weight). Corresponding flours had cadmium concentrations from 0.05 to 0.10 ppm. Cadmium concentrations in 10 consumer products based on wheat were from 0.03 to 0.22 ppm dry weight. Corrections to wet weight would probably lower the values by about 10 percent. The values given may be somewhat high. When the atomic absorption method was compared with the conventional dithizone method, the latter showed values about 40 percent lower.

There have been several investigations of the cadmium content in fish. By analysis of 19 whole fishes from the Great Lakes, Lucas, Edgington and Colby, 1970, found an average cadmium concentration of 0.094 ppm wet weight (neutron activation analysis). Analysis of livers from 10 different species of fish showed mean values from 0.06 to 1.4 ppm wet weight. In an extensive study from New York State, Lovett et al., 1972 examined 406 fishes from 49 freshwaters by atomic absorption technique. The majority of samples of fish meat contained 0.02 ppm or less, and only in a few cases were concentrations above 0.1 ppm found. In the trout, for instance, there was no relationship between age and cadmium concentrations. Havre, Underdal and Christiansen, 1972, determined cadmium in meat of fish from a Norwegian fjord, thought to be polluted by cadmium. Analyses were performed by atomic absorption spectrophotometry after extraction in an organic solvent. Cadmium concentrations in 21 samples of cod ranged from 0.001-0.041

ppm, in 5 samples of pollack from 0.002-0.008 ppm and 14 samples of flounder from 0.005-0.024 ppm wet weight. In 10 cods, cadmium in liver ranged from 0.109-2.225 ppm wet weight. In short, these three investigations on fish show that cadmium concentrations in fish meat usually are very low, whereas in internal organs considerably higher concentrations may be found.

Taylor, 1971, reports on cadmium concentrations in samples of fish and fish products from Great Britain. Although the results are difficult to interpret because the analytical method is not mentioned, the following data are given. Cadmium concentrations in 48 samples of tuna fish were all below 0.2 ppm, but some salmon meat was reported to contain more than 3 ppm. The highest concentrations, up to 5.3 ppm, were found in fish paste.

Reynolds and Reynolds, 1971, examined the cadmium content of crabs (dithizone method). Cadmium concentrations in the white meat were relatively low, 0.02-1.1 ppm, while dark body meat contained from 2.5 to 8.6 ppm of cadmium wet weight.

The cadmium content of German wine was investigated by Eschnauer, 1965, (polarographic method), who found a mean of 3.1 ppb (1.3-4.1) in 13 wines. Bergner, Lang and Ackermann, 1972, (atomic absorption after extraction in organic solvent) found a mean cadmium concentration of 2.9 ppb (0.5-8) in 22 wines and 71 and 40 ppb, respectively, in 2 wines from 1965. Essing et al., 1969, (atomic absorption after extraction in organic solvent) found means of 17 and 8 ppb in 4 red wines and 4 white wines respectively.

3.1.5 Cadmium in tobacco products

In CITE it was mentioned that cigarettes may contain 1-2 ppm of cadmium, and that part of the cadmium could be released into the mainstream and inhaled. Further data supporting cigarettes as an important exposure source have been given by Menden et al., 1972. In American cigarettes they found amounts of 1.56-1.96 µg/cigarette (atomic absorption spectrophotometry). By use of a smoking apparatus (35 ml-puff, 2 seconds every minute) it was found that the particulate phase of the mainstream contained 0.10-0.12 µg/cigarette. For a smoker of 20 cigarettes daily, this means an inhalation of about 2 µg cadmium per day.

Confusing results have been reported by Tomita, 1972, who studied 12 different Japanese brands with a smoking machine. Instead of measuring the amount of cadmium in the mainstream, he mentions that he collected and analyzed cadmium in the sidestream in three bottles. He also measured the amount of cadmium in the whole cigarette, butt and ash. Through subtraction he got the amount in the mainstream. Tomita states that between 30-50 percent of the cadmium in the cigarette may be inhaled by the smoker, which is a figure far above other reported data. The possibilities for serious methodological errors are obvious. The data on the amount of cadmium in whole cigarettes are between 1.35 and 2.5 µg, in accord with data from other countries.

Cadmium content in snuff has been determined (Baumslag, Keen and Petering, 1971). American snuff contained 0.7-0.9 ppm of cadmium and snuff used in South Africa contained 1.1-1.5 ppm of cadmium. This latter type of snuff is a mixture of powdered tobacco with ash of incinerated plants and herbs. The method used was atomic **absorption.**

3.2 DAILY INTAKE OF CADMIUM

In CITE it was concluded that the daily intake in uncontaminated areas is about 50 µg. This estimate was based on data from some countries in Europe, the United States and Japan. This value may be compared with a tolerable weekly intake of 400-500 µg, i.e. about 70 µg/day, proposed recently by a joint FAO/WHO expert committee on food additives (World Health Organization, 1972).

Fukushima, 1972, has calculated that in a non-polluted area of Japan the average daily intake would be 47 µg. Concerning daily intake in polluted areas of Japan, see sections 6.2.5 and 6.3.

In CITE daily intake was discussed based on only data on cadmium concentrations in food and raw material. It is possible to use another approach. The amount of cadmium excreted in feces would consist of unabsorbed ingested cadmium to 90-95 percent, meaning that the fecal excretion would correspond approximately to the daily intake of cadmium.

In CITE it was reported that in one study in West Germany covering 23 persons Essing et al., 1969, estimated the daily fecal excretion to be 31 µg. Tipton and Stewart, 1970, in a long-term balance study of 3 American men found a mean fecal amount of 42 µg Cd/day. Tsuchiya, 1969, referred to Japanese data from a non-contaminated area showing an average daily amount of 57 µg in feces from 4 men. These data for the most part confirm the figure of 50 µg given above for the daily intake. There is a tendency for a somewhat higher value in Japan and a somewhat lower one in Germany.

Unfortunately no methodological studies on cadmium analysis in feces have been reported. There is reason to believe that feces analyses are rather reliable even without extraction methods since interfering salts are not present to the same extent as in urine.

3.3 CONCLUSIONS

Cadmium concentrations in ambient air vary from less than 0.001 µg/m^3 in non-polluted areas to several tenths of a microgram in areas near cadmium emitting industries. Moss analyses have indicated that in one part of Sweden dust fall of cadmium has increased during the last 50 years.

Cadmium concentrations in both natural and drinking water are usually less than 1 ppb. The uptake of cadmium in grains, such as wheat, is influenced by the pH and the cadmium concentration of the soil. The use of sludge may increase the cadmium uptake in wheat grains severalfold. The available data on wheat are from laboratory studies and field studies are needed.

Analyses of cadmium in foodstuffs have shown that cadmium concentrations generally are less than 0.05 ppm. In marine organisms, fish meat contains very small amounts of cadmium, whereas internal organs of fish and shellfish contain appreciable amounts.

The previous estimate of a daily intake of 50 µg cadmium in non-polluted areas still seems to be the best average estimate, granted a possible regional variation from 30-70 µg.

TABLE 3:1 Cadmium uptake in wheat in relation to added sewage sludge, cadmium concentration in soil and pH of soil. Means of four replicates. Atomic absorption, ppb dry weight. (From Linnman et al., to be published).

CaO added (%)		0		0.1		0.2	
Sludge ton/ha	Added Cadmium in soil ppm	pH	Cadmium uptake ppb	pH	Cadmium uptake ppb	pH	Cadmium uptake ppb
0	0	4.8	67	6.1	45	7.4	29
6.5	0.031	4.8	119	6.1	86	7.2	33
19	0.094	5.1	170	6.2	123	7.2	50
58	0.28	5.3	257	6.2	134	6.8	86
175	0.84	5.8	124	5.9	147	6.5	122

TABLE 3:2 Cadmium content ppm wet weight in different food categories in USA. Total number of samples: 30. (From Corneliussen, 1970; Corneliussen, in press).

	Cadmium ppm wet weight			
	1968-1969		1969-1970	
Type of food	No. ≥0.01	Maximum	No. ≥0.01	Maximum
Dairy products	10	0.09	9	0.01
Meat, fish and poultry	21	0.06	22	0.03
Grain and cereal products	27	0.08	27	0.06
Leafy vegetables	27	0.08	28	0.14
Legume vegetables	16	0.03	10	0.04
Root vegetables	24	0.08	27	0.08
Garden fruits	25	0.07	27	0.07
Fruits	15	0.38	10	0.07
Oils, fats and shortening	27	0.13	28	0.04
Sugar and adjuncts	18	0.07	27	0.04
Beverages	8	0.04	9	0.04
Potatoes	-	-	29	0.08

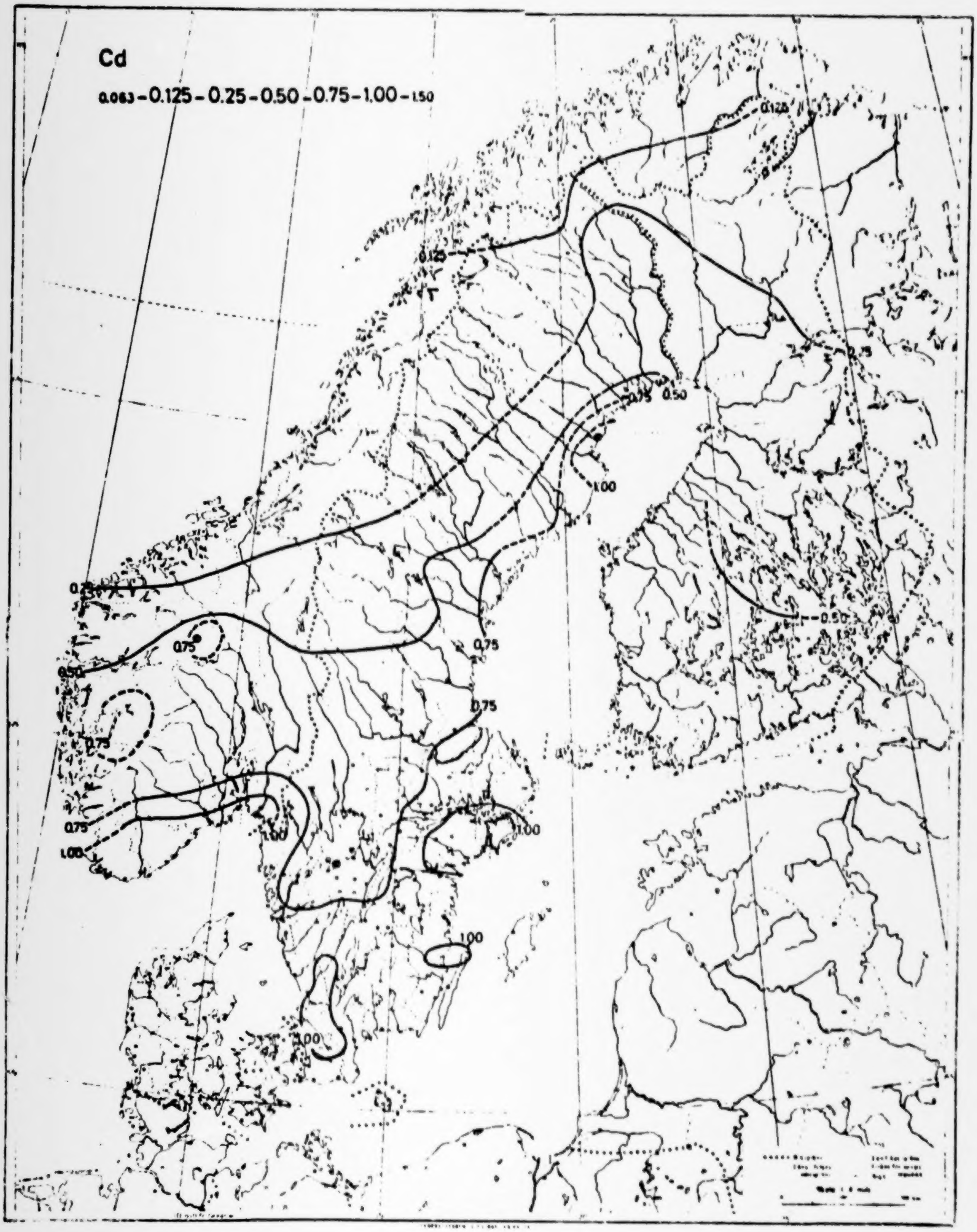

Cd
0.063-0.125-0.25-0.50-0.75-1.00-1.50
0.125
0.125
0.75
0.50
0.75
1.00
0.25
0.50
0.75
0.50
0.75
0.75
0.75
0.75
1.00
1.00
1.00
1.00
1.00

FIGURE 3:1 Isocurves for cadmium (ppm dry weight) in moss in different parts of Scandinavia. Broken lines = values uncertain. (From Rühling and Tyler, 1972).

CHAPTER 4 METABOLISM

At a recent international symposium on accumulation of toxic metals (Task Group on Metal Accumulation, 1973) general aspects on absorption, transport, distribution, excretion and accumulation of metals were discussed.

The term "absorption" was defined as "entry into the body by passage of the metal compound across a membrane". According to this definition, e.g. metal taken into the cells of the mucous lining of the gastrointestinal tract should be considered as absorbed metal, even if the metal passes again out into the gastrointestinal lumen and out with feces, without ever reaching the circulation. The term "systemic absorption" was never used. The term "absorption" in the following text will mean the amount or fraction of metal entering the circulation (i.e. systemic absorption).

The amount of metal found in feces derives from the fraction of ingested material, that passes unabsorbed through the gastrointestinal tract as well as from net gastrointestinal excretion. Net gastrointestinal excretion is defined as total gastrointestinal excretion minus the amount of metal reabsorbed. Gastrointestinal excretion in this context also includes possible excretion by glands in the upper parts of the alimentary tract.

In the following the terms defined above concerning various types of gastrointestinal absorption and excretion will be used.

4.1 UPTAKE AND ABSORPTION

In our previous review (Cadmium in the Environment, = CITE) it was concluded that cadmium is absorbed to a considerable degree after inhalation. Absorption is primarily from the lungs, but to some extent also from the gastrointestinal tract after mucociliary clearance. Human data on absorption were not available but animal experiments suggested an absorption of between 10-40 percent of inhaled cadmium. Results from new studies are not available and it is therefore impossible to give any further data on the fate of inhaled cadmium. Further proof that smoking will cause inhalation and absorption of cadmium will be given in section 4.3.5.

With regard to gastrointestinal absorption, animal experiments indicated that a few percent of orally administered cadmium was absorbed and studies on one human being indicated an absorption of about 6 percent of ingested cadmium. Furthermore it was shown that dietary factors influenced the absorption of cadmium. Especially a low intake of calcium resulting in calcium deficiency would increase this uptake.

4.1.1 Gastrointestinal absorption

4.1.1.1 In animals

The absorption of orally administered cadmium (1.7 and 0.17 mg Cd/kg body weight) in four monkeys was reported in CITE. The retention 10 days after exposure was 2.5 to 3.2 percent. Two additional monkeys exposed to a single dose of only 1 μg/kg body weight have now been studied (Nordberg, unpublished data).The retention after 10 days was of the same magnitude, 2 and 3 percent. This retention is considerably lower than the one reported by Suzuki et al., 1971, only 5 days after a similar exposure in a monkey. They found about 20 percent of the dose in the gastrointestinal tract at that time. These results may indicate that a considerable amount of unabsorbed cadmium will pass out via feces as late as between the 5th and the 10th day.

Ogawa et al., 1972a, gave mice a single oral dose of 1 µCi of $^{115m}CdCl_2$. The whole body retention after 24 and 48 hours was 7.3 and 2.7 percent respectively. As gut contents will be included in these figures, it can only be concluded that the retention was less than 2.7 percent after 48 hours, which agrees with earlier data on mice. Differences in retention rates after oral exposure to cadmium sulfate and cadmium stearate were found by Schmidt and Gohlke, 1971. They gave rats by stomach tube 15 mg Cd/kg of body weight of the compounds twice a week for 7 weeks. It can be concluded that cadmium levels in liver after 3,7 and 16 weeks in rats which had received cadmium stearate were only 44, 32 and 51 percent respectively of the levels found in animals given cadmium as sulfate.

In two investigations the influence of calcium deficiency on absorption and retention of cadmium after oral exposure has been described. Kobayashi, Nakahara and Hasegawa, 1971, gave rice containing about 0.1 and about 0.6 ppm of cadmium to mice on low and normal calcium intake. After 70 weeks of exposure, it can be seen that animals on a low calcium diet had liver and kidney concentrations of cadmium 50 percent (mice exposed to 0.1 ppm Cd) to 300-400 percent (0.6 ppm) higher than mice on a normal calcium diet. Piscator and Larsson, 1972, gave rats on low and normal calcium diets cadmium in drinking water in concentrations ranging from 0-10 ppm for 1 year. Calcium deficient animals retained about twice as much cadmium in liver and kidney as those on a normal calcium diet. These two reports give further support that calcium deficiency will increase the absorption of cadmium.

4.1.1.2 In human beings

Rahola, Aaran and Miettinen, 1971, studied the fate of ^{115m}Cd given orally to 5 human male volunteers, age 19-50 years. They received single doses of 4.8 - 6.1 μCi of ^{115m}Cd, mixed with a calf kidney suspension. The total ingestion of cadmium was about 100 μg. During the first 3-5 days after administration about 70 percent of the activity was eliminated, primarily in the feces. A rapid elimination continued until about 6 percent (4.7 - 7.0 percent) of the dose remained in the body. This indicates an average absorption of at least 6 percent.

Kitamura, 1972, reported on two balance studies performed on a 55-year old man. In one study 5 mg of $Cd(NO_3)_2$ was administered in drinking water (10 ppm Cd) during one day. The total fecal amount was collected during 15 days and cadmium excretion thus calculated. During the first six days, Kitamura analyzed cadmium concentrations in urine, which varied between 5 and 17 μg/l. Before the experiment he had found 16.1 μg/l and therefore concluded that urinary cadmium excretion did not play any significant role in the elimination of absorbed cadmium after a single exposure. The absorption of cadmium was calculated as the amount given minus the accumulated amount excreted in feces. In order to establish a background fecal excretion of cadmium, the subject ate rice containing a total of 20 μg cadmium per day from the day before the experiment and for 6 days. Apart from this rice, the subject ate an unidentified amount of other food with low cadmium content (Kitamura, pers. comm.). In this way Kitamura estimated the background excretion to be 20 μg and subtracted this from the measured values. Between the 6th day and the 15th day the subject ate common food. Kitamura calculated that the long-term absorption of this single dose in drinking water would be 5.34%.

In another experiment, Kitamura, 1972, administered about 5 mg of $Cd(NO_3)_2$ in rice to the same person. The experiment was performed in a similar way but no background measurements were made and no urinary excretions evaluated. Using the same background (20 μg/day) a long-term absorption of 1.35% was calculated. Based on Kitamura's data, it can be calculated that, if the fecal background excretion instead had been 40 μg/day, which better corresponds to reported cadmium-intakes in Japan (section 6.2.5), absorption would be about 10%. A doubling of the background gives a sevenfold increase in absorption! A possible error in background is multiplied into larger errors in calculated absorption, when the method by Kitamura is employed.

Alexander, Delves and Clayton, 1972, made balance studies on children. There must have been serious errors in the analytical procedures because they found a mean urinary excretion far above what is seen even in exposed workers. The data cannot be used for an evaluation of absorption of cadmium.

4.1.1.3 Conclusions

New data have confirmed earlier observations both with regard to absorption in animals and in human beings. In male adult human beings the absorption rate is about 6 percent. When there is a great calcium demand or a calcium deficiency, absorption may be considerably higher. In CITE, it was stated that an absorption in humans of about 10 percent of ingested cadmium must be considered quite possible in the individual case. In view of the new data this figure may be even higher.

4.1.2. Placental transfer

Tanaka et al., 1972, gave pregnant mice single intravenous injections of 50 μCi of ^{115m}Cd as chloride (about 15 μg Cd/mouse) 24-36 hours before delivery. The mean uptake in newborns was 0.09 percent of the dose.

4.2 TRANSPORT, DISTRIBUTION AND EXCRETION OF CADMIUM IN ANIMALS

4.2.1 Uptake to and clearance from blood

It was concluded in CITE that cadmium in blood after injection will initially be found mainly in plasma. During the first 24 hours there is a rapid decrease in plasma. After about 24 hours the cadmium content in the red cells increases. It was also concluded that metallothionein plays a role in the transport of cadmium in the red cells.

New data give further proof for the above mentioned cadmium metabolism in blood. Shaikh and Lucis, 1972a, gave rats subcutaneous injections of 2.2 µg ^{109}Cd as the chloride. There was a rapid initial decrease in plasma levels of cadmium 24 hours after injection and an increase in the red cell levels. Cadmium in plasma was associated predominantly with β-globulins. Nordberg, 1972a, gave mice single subcutaneous injections of ^{109}Cd as the chloride corresponding to 1 mg Cd/kg body weight. Cadmium concentrations in cells and plasma were studied from 20 minutes to 96 hours after injection. Figure 4:1 shows an initial uptake in the red cells with a first maximum reached after about 4 hours. There was then a decrease in the cadmium content in the red cells up to 24 hours, and then once again an increase. After 96 hours the concentration was more than twice as high as after 24 hours. In plasma there was a rapid decrease from 20 minutes to 48 hours. After that there was an increase in plasma levels. Nordberg also studied the distribution of cadmium in the red cells by separating hemolysates on G-75 Sephadex columns. After 20 minutes most of the cadmium was in high molecular weight fractions whereas only a minor part was in the fraction corresponding to hemoglobin and an insignificant amount in the fraction corresponding to metallothionein. After 96 hours a redistribution had taken place, so that about 1/3 of the total cadmium was found in fractions corresponding to metallothionein. The main part was still in high molecular weight fractions, and no cadmium was observed in the hemoglobin peak. In plasma, at 20 minutes and 4 hours after injection, the main part of the cadmium was in fractions corresponding to albumin or larger proteins.

4.2.2 Tissue distribution and retention

In CITE it was concluded that cadmium accumulates mainly in liver and kidneys. After a single exposure the main part of the cadmium is found in the liver. With time there will be a redistribution and eventually the highest concentration will be found in the kidneys, especially the cortex. When a single exposure is involved, this redistribution eventually will also cause the cadmium concentration in the pancreas to exceed that in the liver. Before toxic effects, such as renal tubular dysfunction, have occurred repeated exposure will cause a continuous accumulation in organs. Once renal damage has occurred, urinary excretion is increased. This may lead to a decrease in kidney levels.

Shaikh and Lucis, 1972a, gave mice and rats single subcutaneous injections of 1.1 and 2.2 µg ^{109}Cd as chloride respectively. There was a continuous accumulation of cadmium in mouse kidneys during the observation period of 25 days, whereas in the rat kidney accumulation ceased after 10 hours after which renal levels of cadmium decreased. This was explained as a species difference, but the results in rats are in sharp contrast to the results obtained by Gunn and Gould, 1957. They found an increase in renal cadmium levels continuing for months after a single injection in rats. The drop in renal levels after 10 hours is also the opposite from the earlier results obtained in rats by Lucis, Lynk and Lucis, 1969.

Suzuki et al., 1971, gave pregnant as well as non-pregnant mice single subcutaneous injections of 0.0025 µCi ^{109}Cd as chloride (specific activity not stated) per g body weight. The pregnant rats tended to have higher amounts of cadmium in kidneys and liver, whereas concentrations in digestive tract and bones tended to be less than in controls. The authors concluded that pregnancy may alter the metabolism of cadmium.

The metabolic changes after repeated exposure have been studied by Nordberg (unpublished data). The experimental conditions have been described in detail (Nordberg and Piscator, 1972). In Table 4:1 are shown organ concentrations of cadmium in groups of mice exposed to 0.25 mg and 0.5 mg Cd/kg body weight subcutaneously for 6 months. In the group with highest exposure, signs of renal tubular dysfunction, as indicated by tubular proteinuria, and a sharp increase in the excretion of cadmium had become evident 3 weeks before the end of the experiment. It will be seen that in the spleen the cadmium concentration is twice as high in the high exposure group as in the group with lower exposure. This was also the case with other organs, such as intestines, testis, eye etc. Pancreas concentrations are about 50 percent higher in the high exposure group. In contrast, liver and renal levels of cadmium are about the same in both groups.

Nordberg (unpublished data) studied the relation between cadmium in kidney cortex and whole kidney in mice given ^{109}Cd subcutaneously 5 days a week for 3-6 months. In 6 mice given 0.5 mg Cd/day the ratio cortex/whole kidney varied between 1.1 and 1.3. In 5 mice given 0.25 mg Cd/day the ratio varied between 1.0-1.48. A mean ratio in 8 mice exposed for 6 months to 0.025 mg Cd/day was 1.43 (range: 1.1-1.7). The ratios found in mice agree fairly well with ratios found in human beings.

Note that the page Nos 27 and 28 are erroneously omitted and the text continuous on page 29.

The results by Nordberg in Table 4:1 also confirm earlier results by Friberg, 1952, and Bonnell, Ross and King, 1960, who found that during continuous exposure an accumulation of cadmium in pancreas continued after accumulation in liver and kidneys had stopped.

The tissue distribution after long-term oral exposure has been studied by Stowe, Wilson and Goyer, 1972. Rabbits were given 160 ppm as the chloride in drinking water for 6 months. The mean exposure was 15.5 mg Cd/kg body weight per day. Mean cadmium concentrations in liver, kidney, pancreas and spleen were 188, 170, 29 and 10 ppm wet weight respectively. Histopatholcgical examination revealed slight renal tubular damage. The concentrations in kidney and spleen are similar to those found in the above mentioned mice exposed to 0.25 mg/kg by injections, whereas the injection route resulted in higher concentrations in the liver and much higher in the pancreas.

The differences in accumulation rates of liver and pancreas will be of importance when discussing the findings in human beings excessively exposed to cadmium.

With regard to distribution in other organs, only traces of cadmium were found in brain of adult mice after a single injection (Berlin and Ullberg, 1963, Nordberg and Nishiyama, 1972). Some data by Lucis, Lucis and Shaikh, 1972, may indicate that cadmium will more easily penetrate the blood-brain barrier in rats in the fetal stage. As it was not possible to quantitate the uptake in the brain, more data are needed. In the same work newborns exposed to cadmium via milk from the mothers showed a rapid build-up of cadmium concentrations in the intestines, but only a very slight increase in the liver. It is not clear whether the high concentration in the intestines was due to a local uptake in the intestinal walls or to a slow passage of the intestinal contents. Lucis, Lucis and Shaikh, 1972, also found that cadmium accumulated in the mammary glands of pregnant rats but only small amounts were excreted via milk.

4.2.3 Distribution within organs

The subcellular distribution in the rat liver has been studied by Shaikh and Lucis, 1972b, after injection of ^{109}Cd as chloride. 24 hours after injection about 80 percent was in the soluble fraction and about 9 percent in mitochondria and microsomes. Only very small amounts were found in the nuclei. Webb, 1972a, made similar studies on rat kidney cortex at different times after subcutaneous injections of cadmium chloride, 2.4 mg/kg body weight. With time, the cadmium content of the soluble fraction increased, whereas cadmium content of lysosomes and mitochondria decreased. After 15 days cadmium was practically absent from these organelles. There was some increase with time in the microsomes, paralleling the increase in the supernatant.

Lucis, Lucis and Shaikh, 1972, separated mammary gland soluble proteins from cadmium exposed pregnant rats, and found that cadmium was bound to high molecular weight protein and was not found in low molecular weight protein fractions. Placenta, however, contained cadmium both in high molecular weight proteins and in a protein fraction with a molecular weight of the same size as metallothionein.

Nordberg, 1971a, studied the distribution of cadmium among cellular organelles, free cytoplasma and different cytoplasmic proteins in testicles of mice. A homogenate of testicles was ultracentrifuged at 100,000 x g and the supernatant was separated by G-75 Sephadex gel chromatography. A change with time after single subcutaneous injection of ^{109}Cd-tagged cadmium chloride (1 mg Cd/kg body weight) was observed. Four hours after injection 50 percent of testicular cadmium was in the supernatant and only 26 percent of the cadmium in the supernatant was in a small protein, corresponding to the size of metallothionein. The corresponding figures, 4 days after injection were 74 percent in the supernatant and 55 percent in the small protein.

4.2.4 Excretion

4.2.4.1 Urinary excretion

Earlier investigations (Friberg, 1952) have shown that only a very small part of the dose was excreted via the urine provided that there was no renal damage. Attempts were not made at that time to relate excretion to body burden.

The very low urinary excretion of cadmium has been verified by Shaikh and Lucis, 1972a, in rats given a single subcutaneous injection. They found a daily urinary excretion during four days of only between 0.003-0.007 percent of the dose. Similarly, Nordberg and Piscator, 1972, and Nordberg, 1972a, found that during repeated subcutaneous exposures to 0.025 to 0.5 mg cadmium/kg body weight for up to six months the urinary excretion in mice was very low. At the time that pathological urinary proteins were observed at electrophoresis (after 21 weeks) in the group given the highest exposure, there was a sharp increase in urinary cadmium excretion. It was shown by Nordberg and Piscator, 1972, by means of gel filtration, that a large part of the cadmium in urine was bound to proteins with a molecular weight corresponding to that of metallothionein.

Nordberg, 1972a, b, in the studies referred to above, also showed that the urinary excretion on a group basis was significantly related to total body burden (Figure 4:2). At the highest dose level 0.02 percent was excreted daily, whereas at the lower dose levels about 0.01 percent was excreted. However, as seen in Figure 4:3, there is a considerable individual scatter.

The excretion of cadmium compounds in the rat has also been studied by Furst, Cadden and Firpo, 1972. Cadmium in urine was analyzed by atomic absorption spectrophotometry without any special pretreatment for eliminating interferences. The authors calculated that more than 50 percent of the dose was excreted in a few days. This is probably an error due to influence from sodium chloride in the urine as has been discussed in CITE. No conclusions can be drawn from the studies with regard to excretion.

Murata, 1971, gave oral doses of 10 and 20 μCi ^{109}Cd as chloride (carrier free) respectively to mice. During the first day about 25 percent and in 3 weeks about 50 percent of the total dose were excreted with the urine. The total amount of cadmium in feces during 3 weeks was only about 25 percent of the dose. The data do not fit at all with earlier experience (see CITE), as they indicate an extremely high absorption. Furthermore, the sum of urinary and fecal excretion was only about 75 percent of the dose in 3 weeks, which would indicate an extremely high retention. No explanation for these inconsistent results can be given at present, but some methodological error must be suspected.

4.2.4.2 Excretion via the alimentary tract

Shaikh and Lucis, 1972a, found that about 5 percent of a single subcutaneous dose of 2.2 μg ^{109}Cd as chloride was excreted via feces of rats during the first 4 days.

Biliary excretion of cadmium may be of importance (Caujolle, Oustrin and Silve-Mamy, 1971). Rats were given intraperitoneal injections of ^{109}Cd as sulfate. The dose was 7 µCi, specific activity not stated. A relatively constant excretion of cadmium via bile was found up to 6 hours after injection. When the bile duct of one rat was connected to the small intestine of another rat, cadmium excreted via bile was reabsorbed to a certain extent, indicating an enterohepatic circulation. However, they did not present their data in a way that makes it possible to evaluate this quantitatively.

Nordberg, 1972a, did not find any correlation between total body burden and fecal excretion of cadmium in mice. When a larger number of animals were followed in a later study (Nordberg, 1972b), a certain correlation was found. In Figure 4:4 (where individual data are given), the results are seen from a group of mice exposed to 0.025 mg Cd/kg body weight 5 days/week for about 3 months. It is seen from the figure that a considerable individual scatter exists. If a linear regression analysis is carried out, a significant correlation is found ($r = 0.42$, $n = 149$). The regression coefficient is 0.39 and differs significantly ($p < 0.001$) from zero. This would favor that some part of the excretion is dependent on body burden of cadmium. On the other hand, in another experiment in which the daily dose was 10 times higher (Figure 4:5), it could be shown that the fecal excretion at a certain body burden also was about 10 times higher. This means that at the dose levels studied the main part of the excretion must be related to the daily dose. Upon two occasions during the experiment with the lower doses, exposure was withheld for three weeks. Observations made after the exposure-free weeks are shown in Figure 4:4 (unfilled circles). These values consistently fall below the regression line, further supporting a relation between fecal excretion and daily exposure. As the average of these fecal excretion values was higher at the end of the experiment (14.2 µg/24 h S.D. = 11.0) than in the beginning (5.1 µg/24 h S.D. = 3.1), part of the excretion may be related to body burden.

4.2.4.3 Other excretion routes

In CITE, the excretion via hair was mentioned. No further studies on this excretion route are available. There are other excretion routes which may be worth mention. From the data of Lucis, Lucis and Shaikh, 1972, it can be calculated that excretion was less than 0.05 percent per gram milk per day after a single subcutaneous postpartum injection to female rats. Tanaka et al., 1972, gave pregnant mice 50 µCi of ^{115m}Cd (15 µg Cd) in a single intravenous injection 24-36 hours before delivery. In the newborns only 0.09 percent of the dose was found, i.e. the amount excreted via the placenta. The cadmium concentration in milk was not measured. Sucklings born to female mice not given radioactive cadmium were suckled by the female mice which had been given radioactive cadmium before delivery. Whole-body measurements of the sucklings showed that after 14 days 0.3 percent of the dose given the sucklers was found in the sucklings. As some cadmium must have been passed out via the feces of the sucklings, the total amount excreted via the sucklers' milk must have been more than 0.3 percent.

4.2.5 Biological half-time

One of the more important questions with regard to cadmium metabolism is the biological half-time, extensively discussed in CITE. Animal experiments had shown highly varying values for biological half-time of cadmium depending on dose, administration, single or repeated exposure, length of observation period etc. A general discussion of questions on evaluating the biological half-time can be found in a paper by Nordberg, 1972a, and in a report by the Task Croup on Metal Accumulation, 1973.

There is not much new information on biological half-time in animals, but studies by Webb, 1972a, will be discussed. He followed rats for up to 82 weeks after a single subcutaneous injection of about 2.5 mg Cd as chloride per kg body weight. Up to 21 weeks 2 animals were killed each week, and then two animals at 35 and 82 weeks respectively (Figure 4:6). Maximum levels in liver were reached at about 10 weeks after exposure and slightly later in kidneys. Values at 35 and 82 weeks were only slightly lower than at 21 weeks. The interpretation of the results is difficult as the number of animals at each time is only 2. If the data are taken at their face value, they indicate a long biological half-time in rat kidney. In the liver there was a slightly faster clearance.

Webb also studied cadmium concentrations in testis and epididymis. In the former organ there was an increase in cadmium levels up to at least 18 weeks. This may partly be explained by the necrosis and atrophy of the organ, which are induced by the injected dose (2.5 mg Cd/kg). In epididymis maximum levels were obtained after 5 weeks, the concentration had been halved after about 12 weeks and at 82 weeks no cadmium could be demonstrated there. Also in this organ some pathological alterations could be expected from the dose injected.

The results by Webb, 1972a, given above for testis and epididymis differ from those reported by Gunn, Gould and Anderson, 1968a, who also used rats, but gave a non-injurious dose of ^{109}Cd. They found no time-related changes in concentration of cadmium in testis and epididymis from 1 hour up to 6 weeks after injection.

The strikingly slow accumulation in all organs studied by Webb is difficult to account for; one reason may be a retention of the high dose at the injection site.

4.2.6 Influence of other compounds on the metabolism of cadmium

Special attention in this context was paid to selenium and cysteine in CITE. The possible effect of chelating agents was also discussed. Some new studies are now available on the influence of NTA (nitrilotriacetic acid) on the metabolism of cadmium.

Scharpf, Ramos and Hill, 1972, gave female rats single oral doses of $CdCl_2$ and NTA. There is all reason to believe that serious methodological errors were introduced because cadmium in urine was determined with atomic absorption spectrophotometry without previous extraction or other treatment. Because of salt effects the urinary values will be too high under such circumstances. One group of animals was given 64 mg of cadmium chloride per kg body weight, which will correspond to about 10 mg of cadmium to a 250 g rat. The excretion of cadmium in urine after 96 hours was 1.97 percent of the dose in rats given cadmium and NTA and 0.76 percent of the dose in rats given only cadmium. It has repeatedly been demonstrated that in normal rats absorption of cadmium after oral exposure is only about 2 percent, indicating that in this case not more than about 200 µg cadmium per rat was absorbed. An excretion of 0.76 percent of the dose would mean about 76 µg, which is more than one third of the dose, an extremely unlikely figure.

In the same report it was found that in liver and kidneys, where the results of cadmium analysis probably are more reliable than in urine, cadmium concentrations were considerably lower in animals given cadmium chloride and NTA, than cadmium chloride alone. This has also been shown by Scharpf et al., 1972, in another experiment on rats in which cadmium chloride was given without NTA to one group and with NTA, 200 mg/kg body weight to another group. Liver levels of cadmium were more than 3 times higher in the group given cadmium chloride alone. Whether this is due to a lower absorption or to a higher excretion when cadmium is given together with NTA is not known.

Nolen et al., 1972, and Nolen, Bohne and Buehler, 1972, studied the effects of NTA on cadmium metabolism in rats. Nolen et al. gave groups of pregnant rats cadmium as the chloride in drinking water so that the exposure was 0, 0.01, 1 and 4 mg/kg body weight per day. Sodium-NTA was given in water in doses corresponding to 0, 0.1 and 20 mg/kg body weight per day. Exposure time was between days 6-14 of the gestation period. On day 21 the animals were killed. At all cadmium exposure levels there were no differences in cadmium content of liver and kidneys between animals without or with NTA. In the study by Nolen, Bohne and Buehler, pregnant rats were studied under similar conditions as above. Cadmium was given in oral doses of 4 mg/kg/day. Na_3NTA was given in a dose of 20 mg/kg/day and also in that dose together with $FeCl_3$ (7 mg per kg/day). One group was given sodium citrate (20 mg kg/day). This time, rats given NTA or NTA + Fe had significantly higher cadmium levels in liver than animals given only cadmium. Citrate also caused higher liver levels. In kidneys both NTA and citrate caused higher cadmium levels.

Nordberg, 1971b, gave 0.1 percent NTA in the drinking water for 3-4 weeks to one group of 4 mice and ordinary tap water to another group of 4 mice. Both groups were given 7 µg Cd/kg in the form of $^{109}CdCl_2$ via stomach tube on the third day of the experiment. The body burden of cadmium as well as the excretion of cadmium in urine and feces was followed by measurements of radioactivity. Three days after the cadmium exposure, the NTA-group had retained 9.7 percent of the administered dose and the other group 18 percent. Seven days after dosing, the corresponding figures were 1.9 and 1.8 percent, respectively and 14-18 days after dosing 1.6 and 1.6 percent respectively. The mice were killed 24-29 days after dosing. The mean liver concentration of cadmium in the NTA-exposed group was 0.53 ng/g (range 0.27-1.05) and in the non-exposed group 0.76 ng/g (range 0.24-1.53). Kidney concentrations were 1.41 ng/g (range 0.92-2.70) in the NTA-group and 1.62 ng/g (range 0.76-2.38) in the control group. In short, no large difference in organ retention was found.

Nordberg, 1972c, also studied liver and kidney retention of radioactive cadmium in mice which had been given a single subcutaneous injection of $^{109}\mathrm{CdCl_2}$ (0.1 µg Cd/kg body weight). They then drank water containing 50 ppm non-radioactive cadmium for several months. Half of the 38 mice in the study were also exposed to 500 ppm NTA in the drinking water. At the end of the observation period (4 months) 14 mice in the cadmium-NTA group were alive versus 11 in the control group. The mean concentration of radioactive cadmium in liver and kidney of the surviving mice was as follows: Group Cd + NTA: Liver 1.18 ppm (wet weight); kidney 0.98 ppm. Group Cd: Liver 1.33 ppm; kidney 1.21 ppm. That is to say, no large differences in cadmium concentrations were observed.

4.3 TRANSPORT, DISTRIBUTION AND EXCRETION OF CADMIUM IN NORMAL AND EXPOSED HUMAN BEINGS

4.3.1 Transport and distribution in blood

Reviewing data from various countries, it was concluded in CITE that the average normal cadmium level in whole blood in normal human beings is well below 1 µg per 100 ml whole blood. The main difficulty in assessing normal values has been that the analytical methods have not yet been so perfected so as to measure accurately these small quantities of cadmium in blood. To date, no accurate studies on analytical methods have yet been carried out.

Vens and Lauwerys, 1972, found a mean concentration of 0.95 µg/100 ml whole blood (S.D. 1.0) in 24 normal subjects. (See also Chapter 2). In the red cells a mean concentration of 2.16 µg/100 ml (S.D. 2.69) was reported. The concentrations in whole blood are somewhat higher than those earlier reported. It is not quite clear from the paper whether the values for cadmium in red cells were obtained by actual determinations or by the assumption that all cadmium was in that fraction and the use of hematocrite values.

Another recent report on normal values is by Delves, Bicknell and Clayton, 1972. They found a mean value of 0.49 µg per 100 ml (atomic absorption after extraction, in organic solvent) of whole blood in healthy children as compared with 0.57 in a group of children admitted to a hospital. The highest individual value noted in the group of patients was 7.9 µg per 100 ml. Such a high value has only been seen in cases of heavy occupational exposure to cadmium. Cadmium concentrations in serum have been determined in patients with and without hypertension by Mertz, Koschnick and Wilk, 1972. In CITE a report by Mertz et al., 1968, was discussed and it was concluded that the values presented were too high for cadmium in serum. The same can be said of the report on the hypertensive patients. Mertz, Koschnick and Wilk, 1972, obtained a mean cadmium concentration in serum of 23 µg/100 ml, and the maximum value was 80 µg/100 ml. As this is a value which considerably exceeds even concentrations obtained in experimental studies involving radioactive cadmium isotopes on severely poisoned animals, some serious analytical errors must have been involved. (See also section 4.3.4.1, where this method gave a urinary cadmium excretion of almost 1 mg/day).

There are also some new data on cadmium in the blood of exposed workers. Piscator, 1972, studied a group of 25 workers in an alkaline battery factory and related cadmium concentrations in blood (spectrographic method, "normal" concentration < 0.2 µg per 100 ml) to exposure time but found no relationship (Figure 4:7). Short exposures could be combined with high concentrations in blood and long-term exposure with low concentrations of cadmium in blood. In another study (Piscator, unpublished data) it was possible to follow the decline in blood levels of cadmium in a group of about 20 people, who for 1-2 years must have been very heavily exposed to a cadmium aerosol during polishing of cadmiated metal. Extremely high levels of cadmium were found in blood, after which the operations were closed down and the workers moved to another workshop. Cadmium in blood was determined 3 and 9 months after cessation of exposure (Figure 4:8), and during that period the half-time of cadmium in blood was about half a year.

4.3.2 In organs

Available data on concentrations of cadmium in organs, especially liver and kidneys were presented in CITE. In some European countries and the United States, liver concentrations in adults were between 1 and 3 ppm wet weight, whereas in one area in Japan, regarded as not cadmium contaminated, levels in adults between 50 and 75 years of age reached above 10 ppm. In renal cortex levels of 20-30 ppm wet weight at age 50 were found in the United Kingdom and Sweden, whereas in the United States, the corresponding value was about 50 ppm. Higher values were reported from Japan, where in one area classified as non-polluted, an average level of 90 ppm was reached at age 50.

Some new data on kidneys have now been obtained and are presented in Figure 4:9. At around age 50 cadmium levels in cortex from men in East Germany are 25-30 ppm wet weight, whereas in women from the same country the concentration is around 15 ppm (Anke and Schneider, 1971). Corresponding figures for men and women in the United States (North Carolina) in a new study by Hammer and Finklea, 1972, are 26 and 24 ppm wet weight. Even in other age groups the difference between levels in men and women was not as great as in the report from East Germany. In contrast to these data, Tsuchiya, Seki and Sugita, 1972, (Figure 4:10) reported a mean level around 125 ppm wet weight at age 50 in Japanese (the group consisted of both men and women) from the Tokyo area which in Japan is regarded as a non-contaminated area.

In Figures 4:9 and 4:10 data by Kitamura, Sumino and Kamatanni, 1970, Ishizaki, Fukushima and Sakamoto, 1970, and Schroeder and Balassa, 1961, are also included, together with data on Swedish workers as reported in CITE.

The new data confirm the earlier reported results. In Europe average cadmium concentrations in renal cortex at age 50 will be 20-30 ppm and in so-called non-contaminated areas in Japan, up to 125 ppm. All the group averages from Japanese areas are higher than corresponding values from Europe and USA.

The linear increase as described in CITE has been confirmed. Earlier data from the USA showed a renal cortex level of about 50 ppm at age 50, whereas lower values now have been reported in other studies. Whether regional differences in relation to exposure or other factors may explain the differences is not known.

4.3.3 Distribution in organs

Further information on the distribution of cadmium in kidneys has been obtained by analysis of serial sections (Livingston, 1972). From the outer layer of the cortex and inward, 8 horizontal layer sections, 4 cortical and 4 medullary, were cut and analyzed for cadmium, zinc and mercury by neutron activation analysis. An example is shown in Figure 4:11. The concentration of cadmium was found to decrease from the outer layer inward. In the illustrated case, the content in the outer cortex was about twice as high as in that in the inner cortex nearest the medulla. These data clearly show the necessity of standardizing sampling methods.

4.3.4 Excretion

4.3.4.1 Urinary excretion

In CITE it was concluded that normal excretion of cadmium via the urine is very low, around 2 µg/day or less.

Suzuki and Taguchi, 1970, studied 38 men with a mean age of 29 years (18-44 years) and 49 women, mean age 27 years (16-49 years) from the Tokyo area. They found a mean excretion of 2.38 µg/l in the men and 1.97 µg/l in the women, but did not find any relationship between age and excretion of cadmium. Katagiri et al., 1971, determined cadmium in urine in 303 persons (males and females, ages 4-59) from Gifu in Japan. At ages 4-6 the average urinary concentration was 0.47 µg/l, in age group 30-35 1.13 µg/l and in age groups 40-49 as well as 50-59 1.75 µg/l. Similar results were obtained by Tsuchiya and Sugita, 1971, and Tsuchiya, Seki and Sugita, 1972a, who studied 609 inhabitants of Tokyo (Figure 4:12). From age 5 to 35 years there is an increase in cadmium excretion from about 0.5 to 2 µg/l. Then the excretion is of about the same magnitude to age 55 after which it falls slowly off. Piscator, 1972, found in 10 men, ages 34-63 years, living in a polluted area in Sweden, a mean concentration of 2.1 µg/l. In CITE it was mentioned that in urines from 10 persons in Stockholm, Sweden, 20-40 years of age, the average cadmium excretion was 0.4 µg/day, corresponding to a concentration of 0.34 µg/l (0.07-0.63). All determinations reported here have been made by atomic absorption spectrophotometry after extracting with a chelating agent into an organic solution.

There are also two reports by McKenzie (1972a, 1972b). These data cannot be used because cadmium was determined with atomic absorption without extraction, giving extremely high values. The mean excretion was reported to be 36 and 33 µg Cd/24 hours in men and women respectively. Mertz, Koschnick and Wilk, 1972, have reported on cadmium excretion in hospitalized persons, with and without hypertension. They used a spectrographic method and found a mean excretion of 0.63 µg Cd/min, which would correspond to a mean daily excretion of 913 µg, which is much higher than ever reported in groups of cadmium workers. This is the same method that gave erroneously high results for cadmium in serum.

One main difficulty in evaluating urinary excretion of cadmium in exposed workers as described in much of the data at hand is the fact that it has not always been made clear whether or not tubular dysfunction also existed, which could have caused an increased excretion. This problem was mentioned in CITE and Piscator, 1972, has taken some steps to clarify it. He studied urinary excretion of cadmium in workers exposed to cadmium oxide dust in an alkaline battery factory. In 27 workers and 10 controls urinary cadmium, total urinary protein and urinary β_2-microglobulin were determined and electrophoretic separations of urinary proteins were performed. All analyses were blind, without knowledge of exposure conditions. Even in cases with long exposure time (>5 years) but without signs of renal dysfunction, urinary cadmium generally was below 10 µg/g creatinine. In workers with signs of renal dysfunction as indicated by changes in electrophoretic patterns and increases in β_2-microglobulin, urinary excretion of cadmium was higher (Figure 4:13). It seems that in that type of industry, where concentrations of cadmium in air during the last ten years usually had been around 0.05 mg Cd/m^3, cadmium excretion could not be related to body burden, whereas renal dysfunction caused an increase in the excretion.

There are many difficulties in interpreting these data, however, especially since it is difficult to estimate the individual exposures.

In the study by Piscator (unpublished data) referred to in section 4.3.1, high concentrations of cadmium in blood as well as in urine were observed in workers after 1-2 years of exposure to high concentrations of cadmium. After exposure had ceased, large individual variations were found and no regular patterns were seen (Table 4:2). In some cases there were no important changes with time while in other cases considerable decrease or increase in urinary cadmium excretion was observed. In most cases protein patterns were normal. Total protein excretion was in all cases within the normal limits but as seen in Table 4:2, the relation between different proteins varied. Pathological patterns were sometimes seen on paper electrophoresis.

According to Tsuchiya, Seki, and Sugita, 1972b, protein excretion will increase first at a cadmium concentration of about 50 µg/l of urine. Such a conclusion does not seem to fit with the data presented above by Piscator, 1972. Tsuchiya and collaborators studied only total protein content. It should be pointed out that protein concentrations in urine may well be within normal limits coincident with slight tubular dysfunction, as shown by increases in β_2-microglobin and changes in electrophoretic patterns. Piscator studied such early signs of tubular dysfunction. Furthermore, in the Swedish study the workers were exposed to cadmium oxide dust, while in the Japanese study the workers were exposed to cadmium oxide fumes. The Japanese workers had very high blood levels, highest around 40 µg/100 ml. The study by Tsuchiya, Seki and Sugita, 1972b, would be more comparable with the other study by Piscator (unpublished data) in which the exposure was heavy but over a fairly short period of time. The blood values were high and high amounts of cadmium were excreted in the absence of signs of renal dysfunction. Nomiyama, 1971a, found 5.8 (1.9-13.7) µg Cd/l in the urines of 9 persons without proteinuria (Trichloracetic acid method) compared with 19.8 (17.3-21.2) µg Cd/l in 3 persons with proteinuria.

Sudo and Nomiyama, 1972, studied urinary excretion of cadmium in a 61-year-old male so-called cadmium worker with proteinuria varying between - and ++. Cadmium excretion as measured in three daily urine specimens was studied during one month and showed marked variation (10-50 µg Cd/24 h). Spot samples were also studied for six months. The authors pointed out the necessity for expressing excretion values on a per-day basis rather than on a per volume basis.

Summing up, there are data which may seem to contradict each other. Japanese studies on people not occupationally exposed to cadmium show an increase in cadmium excretion on a group basis which seems to be related to body burden. A similar association with exposure could not be found in Swedish workers exposed for several years to cadmium oxide dust and without signs of tubular dysfunction. Likewise, in workers having undergone a short but very heavy cadmium exposure and having high blood and urinary values of cadmium, the urinary excretion after cessation of exposure did not follow body burden in a predictable way based on the Japanese data. Exposure situations thus seem to be of paramount importance.

4.3.5 Total body burden and renal burden

In CITE it was concluded that the body burden at age 50 in non-contaminated areas in Europe was 15-20 mg, in the U.S. 30 and in Japan 40-80 mg.

Tsuchiya, Seki and Sugita, 1972a, calculated total body burden and renal burden in autopsy studies from Tokyo. The total body burden was assumed to constitute double the amount found in liver and kidneys. At 10-30 years of age, total body burden was about 30 mg, at 30-50 years about 45 mg and at the age of 50-59 about 55 mg. The renal burden at age 50 was about 20 mg. In CITE it was concluded that total body burdens in Europe and the United States were about 15-30 mg at age 50 and so the data by Tsuchiya, Seki and Sugita give further support to the statement that even in so-called non-polluted areas in Japan body burdens of cadmium are considerably higher. The difference will be even more evident if body burden is expressed as mg/kg body weight.

In CITE it was calculated that smoking a pack of cigarettes a day for 35 years might result in 30 percent higher renal cortex levels of cadmium in smokers than in non-smokers. Lewis et al., 1972, determined cadmium in kidney, liver and lung in 45 male smokers (mean age 60, S.D. 11 years) and in 22 male non-smokers (mean age 60, S.D. 12 years). It was possible to calculate the number of "cigarette pack years" for each smoker. In non-smokers cadmium in kidney, liver and lung was on an average 6.6 mg while in smokers the corresponding figure was 15.8 mg. A significant association was found between number of pack years and cadmium accumulation (Figure 4:14) also when age was controlled for.

4.3.6 Biological half-time

It is evident that the data covered in CITE spoke in favor of an extremely long biological half-time (decades) for cadmium. This conclusion was based on a model for cadmium accumulation in the body implying that one third of the body burden was in the kidneys. The great need for further studies on the biological half-time was pointed out.

In section 4.1.1.2 the details are given of an investigation by Rahola, Aaran and Miettinen, 1971, in which radioactive cadmium was given to male volunteers. The decrease in whole-body retention of radioactive cadmium was very slow from 20 days to 2 months after exposure. It was not possible to give an exact value for the biological half-time for this slow component. The shortest estimate was approximately 130 days; the longest one was infinity.

Intermittently during six months, Sudo and Nomiyama, 1972, measured urinary cadmium excretion from a so-called former cadmium worker who had proteinuria (see section 4.3.4). Calculating the biological half-time, they arrived at a value of about 200 days. The authors did not account for the uncertainty in the value, which must have been considerable because of the reported marked variations in the daily urinary excretion and the short observation period compared to the biological half-time. These drawbacks along with the facts that the person had proteinuria and that his fecal cadmium excretion was not taken into account render it impossible to consider the value given by Sudo and Nomiyama as valid.

In some recent papers various mathematical models have been discussed, in some cases based on observed cadmium concentrations in autopsy materials.

Calculations of the biological half-time of cadmium based on autopsy data and calorie consumption were performed by Kjellström, 1971, Tsuchiya and Sugita, 1971, and Tsuchiya, Sugita and Seki, 1972. Biological half-times of from 16 years (Tsuchiya and Sugita, 1971) to 33 years (Kjellström, 1971) have been suggested. Tsuchiya, Sugita and Seki, 1972, calculated the biological half-time to be 17 years for the kidneys and 6.2 years for liver. The basis for the calculations were data on renal and liver burdens of cadmium obtained by autopsies on inhabitants in Tokyo. Hammer and Finklea, 1972, estimated the biological half-time for the whole body to be 9-18 years. They reached this value by using the same model and the same intake figures as given in CITE and applying these to some new autopsy data (section 4.3.2, 25 ppm at age 50).

Both in the paper by Kjellström, 1971, and by Tsuchiya, Sugita and Seki, 1972, it was pointed out that several parameters must be taken into account when calculating the biological half-time, such as changes in food intake, changes in kidney weight with age. In an elaboration on this point, Kjellström and Friberg, 1972, showed how various types of changes in exposure over the years can affect accumulation (as seen in cadmium concentrations in organs). A general discussion of various models used for calculations of toxic metals has been given by Nordberg, 1972d, and by the Task Group on Metal Accumulation, 1973).

Conclusion

The question of the exact biological half-time of cadmium in the human body is still under discussion. All data continue to favor a very long biological half-time.

4.4 RELATIONSHIPS AMONG CONCENTRATIONS OF CADMIUM IN BLOOD, URINE AND ORGANS

4.4.1 Relationships between concentrations of cadmium in blood and in organs

4.4.1.1 In animals

In CITE it was stated that more data were needed before any definite conclusions could be drawn. There are now some additional data available. Nordberg, 1972a, found that if animals were injected subcutaneously for 3 weeks by small doses of cadmium chloride and then exposure ceased, blood levels during 3 weeks decreased with about 40 percent. Whole body burden did not decrease noticeably, indicating that there was no relationship between blood levels and body burden.

4.4.1.2 In_human_beings

In the investigations mentioned in section 4.3.1 (Piscator, 1972) it was found that there was no relationship between cadmium levels in blood and exposure time. Nor was there any relationship between degree of proteinuria and blood levels. No relationship could be found between levels of cadmium in blood and urinary excretion of cadmium. In other investigations in which there had been short-term high exposure to cadmium, a relationship between urine and blood values seemed to exist (Tsuchiya, Seki and Sugita, 1972b).

4.4.2 Relationships between concentrations of cadmium in urine or feces and organ or blood concentrations

4.4.2.1 In_animals_

From the data evaluated in CITE, it seemed as though the excretion of cadmium was dependent primarily upon the daily dose and only to a limited extent reflected total body burden. Nordberg, 1972a,b, has now shown that there is a relationship in mice on a group basis between urinary excretion of cadmium and total body burden, before renal damage has occurred (4.2.4.1). The excretion via feces is dependent on the daily dose but a small part of it also may be dependent on body burden (4.2.4.2).

4.4.2.2 In_human_beings

Concerning fecal excretion, in CITE no data which could show to what extent a fecal excretion of cadmium takes place were quoted. To date, there are still no data available to give any grounds for such a discussion.

In CITE it was stated that the main part of the cadmium excretion via urine was unrelated to renal accumulation but related to the daily dose. There are still no conclusive data which could show whether or not a relation to renal burden exists. The Japanese studies referred to in section 4.3.4.1 (Katagiri et al, 1971, and Tsuchiya, Seki and Sugita, 1972a) show a relation to total body burden on a group basis during long-term low exposure to cadmium. Due to the wide individual scatter the predictive value of urinary cadmium as an index of total body burden on an individual basis is low. Furthermore, data from exposed workers (4.3.4.1) show that in other exposure situations a similar relation with body burden may be absent. There is a great need to study, under different exposure situations, urinary excretion in relation to concentrations in different organs. Under certain conditions and on a group basis it may well be that urinary cadmium is a useful index of accumulation of cadmium.

4.4.3 Conclusions

It was previously stated that no data existed which could show that the concentration of cadmium in blood or urine could be used for estimating organ levels of cadmium in human beings. The investigations, both on animals and normal human beings, discussed in the foregoing sections indicate that in human beings, having a normal renal function and not excessively exposed to cadmium, there might exist a relationship between urinary excretion of cadmium and total body burden on a group basis. Due to a wide scatter, the predictive value is low on an individual basis. It is not known to what extent the excretion is related to the concentration in the critical organ. During exposure situations which may occur in factories, relations between body burden and cadmium excretion may be quite different.

4.5 METALLOTHIONEIN

In CITE some data were given on the unique properties of this metal binding protein, characterized by a low molecular weight, absence of aromatic amino acids, and a high percentage of cysteine in the molecule. Metallothioneins or proteins very similar to metallothionein were thought to play an important role for transport of absorbed cadmium and also for the transport of cadmium to the kidneys.

During the last years many reports both on the occurrence and chemistry of metallothionein have been published. Proteins similar to metallothionein have now been identified not only in liver, kidney and duodenum, but also in other organs of animals exposed to cadmium. Shaikh and Lucis, 1971, found two forms of rat metallothionein, with slight differences in amino acid composition in liver, kidney, spleen and pancreas. Nordberg, 1971a, treated mice with non-radioactive cadmium, 0.25 mg Cd/kg, for 12 days and then gave a high dose of radioactive cadmium 1 mg Cd/kg. In mice not given this pretreatment, the high dose caused testicular damage, whereas the high dose had no effect on the testis of pretreated mice. In mice that had not received any pretreatment with non-radioactive cadmium, it was found that most of the injected radioactive cadmium was in high molecular weight fractions 4 hours after the injection. In contrast, most of the radioactive cadmium in pretreated mice was found in a low molecular weight fraction, corresponding to metallothionein. It was concluded that this was one of the reasons that cadmium did not cause testicular damage at repeated injection. There are also two brief reports by Singh and Nath, 1972, and Chen et al., 1972, on the occurrence of a low molecular weight protein containing cadmium and thiol groups in testis of rats given an intraperitoneal and subcutaneous injection of ^{109}Cd, respectively.

A similar protein was also found in rat placenta, while in the lactating mammary glands of rats, cadmium was only found in high molecular weight protein fractions (Lucis, Lucis and Shaikh, 1972).

Some further data on the chemistry of metallothionein have been provided by Webb, 1972b, who suggested that in contrast to horse liver metallothionein, rat liver metallothionein needed only two SH-groups to bind cadmium. According to Webb these rat proteins are heat resistant, a property which can be used in the preparation of the protein. Webb, 1972b,c, has also thoroughly discussed the evidence behind the claim that metallothioneins are mainly synthesized in response to exposure to cadmium and zinc. Webb could stimulate synthesis of zinc-containing cadmium-binding low molecular weight proteins by giving injections of zinc, which is in contrast to earlier reported results of Shaikh and Lucis, 1970, who could not induce synthesis of cadmium binding proteins by giving zinc. The purity of the zinc compounds given was not stated in either case. As zinc salts may contain cadmium as an impurity, this amount of cadmium may be enough to stimulate synthesis.

Webb, 1972a, when separating liver and kidney supernatants by gel filtration, also found some slight differences in hepatic and renal low molecular weight cadmium binding proteins. He found that they differed in electrophoretic mobilities.

That there are different forms of metallothionein has been shown by Nordberg et al., 1972, who obtained two major forms of rabbit liver metallothionein with different metal contents and different isoelectric points by using isoelectric focusing. The two major forms were then subjected to amino acid analysis, which revealed that the amino acid composition of both forms was very similar and also similar to metallothionein from horse kidney, earlier characterized by amino acid analysis by Kägi, 1970. Some differences between the rabbit liver and horse kidney proteins were notable, i.e. the rabbit protein lacked the arginine, valine and leucine reported in the horse protein.

The two forms of metallothionein purified by Nordberg et al. differed as to their contents of lysine, proline and glutamate. One of these forms contained cadmium and zinc in a molar ratio of 50:1 and the other one in a ratio of 1:1.2. The total molar amount of metal was about the same in both forms. The differences in electrophoretic mobilities and in isoelectric points may be due to both different charges and sizes, dependent on metal content and type of metal, but also on differences in amino acids. A third form, containing only zinc, was also observed, but in smaller amounts, which did not allow further characterization.

More data on horse metallothionein have been given by Roosemont, 1972, who found two metalloproteins in horse renal cortex. These differed in metal content and in ratios between cadmium and zinc.

The ability of metallothionein to bind mercury has also attracted further interest and Wisniewska-Knypl et al., 1972, and Piotrowski et al., 1972, have recently been able to isolate a mercury-binding metallothionein from rat liver. It was shown that the molecular weight was around 10,000, determined by gel filtration and that this protein had the absorption curve typical for metallothionein. Injections of mercury did not induce synthesis of metallothionein to the same extent as cadmium, and in these experiments it was also necessary to inject cadmium to stimulate the production of metallothionein. It has not been shown that exposure to mercury alone causes increases in metallothionein in the liver, but in the kidney an increased amount was found after exposure to mercury (Piotrowski et al., 1972).

Bryan and Hayes, 1972, claim that they found an increase in low molecular weight metal binding proteins in liver of mice after oral exposure to mercury chloride for several weeks. They separated soluble liver components on a short Sephadex

G-75 column (void volume 4.2 ml) at pH 7 and with an eluant of very low ionic strength, and found increases in the 250/280 nm ratio in the low molecular weight fractions, which they estimated to have a molecular weight of 11,000. This estimation cannot be correct. It is difficult to see how reliable molecular weight determinations could be obtained on such a small column without a valid marker procedure. Moreover, a careful examination of the gel filtration patterns in the report reveals that the fraction which is called low molecular weight protein must be composed of compounds probably in the molecular weight range of amino acids.

It should be pointed out that molecular weight determinations, performed by gel filtration, have shown a molecular weight around 10,000 in most studies. Data by Nordberg et al., 1972, confirmed earlier results by Kägi, 1970, that when using the results of amino acid analysis, the true molecular weight of thionein is probably 6,000-7,000.

In a report by Nordberg, 1971a, a pharmacological approach was chosen to study the effects of administration of metallothionein to mice. Graded doses of metallothionein were injected together with cadmium and zinc and it was observed that although the total dose of cadmium was the same in all groups (1.1 mg/kg body weight), animals which were given a certain amount of metallothionein were protected from the testicular damage evident in the group not given any metallothionein. In the metallothionein injected group histological evidence of tubular kidney damage was observed. This is in accordance with earlier theories that cadmium bound to metallothionein will be filtered through the glomeruli.

Regarding metallothionein in human beings our knowledge is still minimal, but some additional information has been obtained from the report by Wisniewska-Knypl, Jablonska and

Myslak, 1971, who investigated a case of acute cadmium poisoning in a man who swallowed large amounts of cadmium iodide (25 mg Cd/kg body weight) in a suicidal attempt. He suffered anuria, among other things. Treatment with a calcium EDTA compound was tried but did not improve his condition and he died after 7 days. Cadmium accumulated mainly in the liver and renal cortex, and it was possible from these two organs to identify low molecular weight cadmium protein complexes by separations on Sephadex G-75. In renal medulla, testis and heart, the main cadmium fraction was also found in a low molecular weight fraction.

From the studies reviewed it is further supported that metallothionein plays an important role in the metabolism of cadmium. There are, however, some points that have to be clarified. It has been shown that in the liver, there are several forms of metallothionein with slightly different properties, due to differences in metal content among other things. In the kidney two forms of metallothionein have also been found. It is not known if the proportion between these two forms is the same in liver and kidney or if there is some selective transport of one of these from the liver to the kidney.

4.6 GENERAL CONCLUSIONS ON METABOLISM

4.6.1 In animals

Uncertainties with regard to absorption rates after inhalation still exist and demand extensive investigations. Further data on intestinal absorption have been provided, showing that both after exposure to cadmium via drinking water and via rice, calcium deficiency will cause a considerable increase in absorption. The initial accumulation of cadmium in red cells followed by a decrease and later by a new accumulation has been confirmed. It has also been shown that this redistribution is connected with a build up of metallothionein in the cells.

Repeated exposure to small amounts of cadmium results in its continuous build up in liver, kidneys and other organs. When renal tubular dysfunction occurs, urinary excretion of cadmium increases. Cadmium levels in the kidneys may even decrease.

The accumulation in liver and kidneys seems to be mainly dependent on the storage of cadmium in the cadmium binding protein, metallothionein. It has been shown that this protein may easily pass through the glomeruli. The urine of exposed animals with tubular proteinuria has been found to contain this protein.

Urinary excretion of cadmium in mice has been shown to constitute 0.01 to 0.02 percent, depending on the exposure levels, of the total body burden before renal damage has occurred. Urinary excretion of cadmium in mice on a group basis is proportional to the total body burden.

4.6.2 In human beings

Further studies on the fate of radioactive cadmium in human beings after ingestion have shown that the earlier reported results for one person, showing an absorption of about 6 percent, were an accurate estimate. In 5 human beings it was shown that 4.7 to 7 percent of the dose was retained in the body. It was estimated in CITE that an absorption of about 10 percent of ingested cadmium must be considered possible under certain circumstances, such as calcium and protein deficiency. Assuming that the influence of calcium deficiency is of the same magnitude in human beings as in animals, it is conceivable that absorption rates even higher than 10 percent may occur.

It was earlier shown that in workers who had been removed from an exposure of several years to relatively low concentrations of cadmium, blood levels decreased faster than body burden. New data indicate that after short-term exposure to high amounts of cadmium, the initial decrease in blood levels is fast.

New data on renal cortex levels from East Germany and "non-polluted" areas in Japan confirmed the earlier trends. Renal cortex levels of cadmium at age 50 are about 20-30 ppm wet weight in Europe. In Japan, in people of the same age, a new study showed a concentration of 125 ppm. New data from other areas in the USA showed levels around 25 ppm, i.e. lower than previously reported from that country.

That in normal human beings the excretion of cadmium via the urine is very low, around 2 µg or less, has been confirmed in several investigations. It was earlier stated, that this urinary excretion was not directly proportional to the increase in total body burden. New data show that the urinary excretion of cadmium increases with age, at about the same rate as the total body burden. This relationship seems to hold true on a group basis for normal populations with moderate exposure to cadmium. A similar relationship has not been found in exposed workers without proteinuria. Furthermore, due to a wide scatter, and based on animal data, the predictive value of urinary cadmium as an index of total body burden on an individual basis is low.

It has also been demonstrated that even when there is only a slight tubular dysfunction as indicated by moderate increases in excretion of low molecular weight proteins, there will be an increase in the excretion of cadmium.

Some estimates have now been obtained for the biological half-time of cadmium in man. Estimates based on mathematical models show that the half-time for total body may be between 10-30 years. There are still considerable uncertainties with regard to biological half-time in total body and different organs, but it is obvious that the biological half-time of cadmium is extremely long. A proper accumulation model for cadmium in different organs, including the kidneys, has yet to be found.

More data have been presented showing that in exposed workers there is neither a relationship between exposure time and cadmium concentrations in blood, nor between cadmium concentrations in blood and urinary excretion of cadmium. The statement in CITE that "the determination of cadmium in blood will be of limited assistance for making estimates of renal concentrations" still seems to hold true.

TABLE 4:1

Cadmium concentrations (ppm wet weight) in liver, kidney, pancreas and spleen in mice exposed to (^{109}Cd) for 6 months (Nordberg, unpublished data)

		liver		kidney		pancreas		spleen	
Dose	n	mean	range	mean	range	mean	range	mean	range
s.c. 0.25 mg Cd/kg body weight, 5 days a week	4	281	271-288	162	141-172	71	67-74	10.1	9.5-10.6
s.c. 0.5 mg Cd/kg body weight, 5 days a week	4	258	190-288	172	158-194	114	111-121	19.5	18.4-21.4

TABLE 4:2 Change in urinary cadmium excretion with time after cessation of exposure (Piscator, unpublished data)

Worker		Urinary cadmium µg/g creatinine					
		time after last exposure =					
Males	Age	2 weeks		10 weeks		8 months	
1	47	32.3	(N)	33.7	(N)	18.1	(N)
2	25	25.4	(G)	39.9	(G-T)	22.3	(G)
3	58	21.9	(N)	18.3	(N)	14.1	(N)
4	63	20.6	(N-T)	34.3	(N)	19.1	(T)
5	53	17.2	(T)	55.0	(T)	22.4	(T)
6	51	13.3	(N)	3.4	(N)	1.9	(N-T)
7	26	10.5	(G)	2.7	(N)	2.9	(N-G)
8	52	4.3	(T)	3.8	(N)	3.7	(N)
9	54	3.7	(N)	3.6	(N)	3.8	(N-T)
10	54	3.6	(T)	11.4	(T)	2.9	(G-T)
11	33	1.2	(N)	1.1	(N)	0.8	(N)
Females							
1	47	72	(N)	55	(N-T)	33	(N)
2	43	17.6	(N)	16.7	(N)	13.0	(N)
3	45	6.3	(N)	4.3	(N)	5.0	(T)
4	43	5.4	(N)	-		6.2	(N)
5	47	2.2	(N)	10.0	(N-T)	3.0	(N)

Letters within parenthesis denote patterns on paper electrophoresi

N = normal
G = glomerular
T = tubular
N-T = suspected tubular
N-G - suspected glomerular
G-T - mixed tubular-glomerular

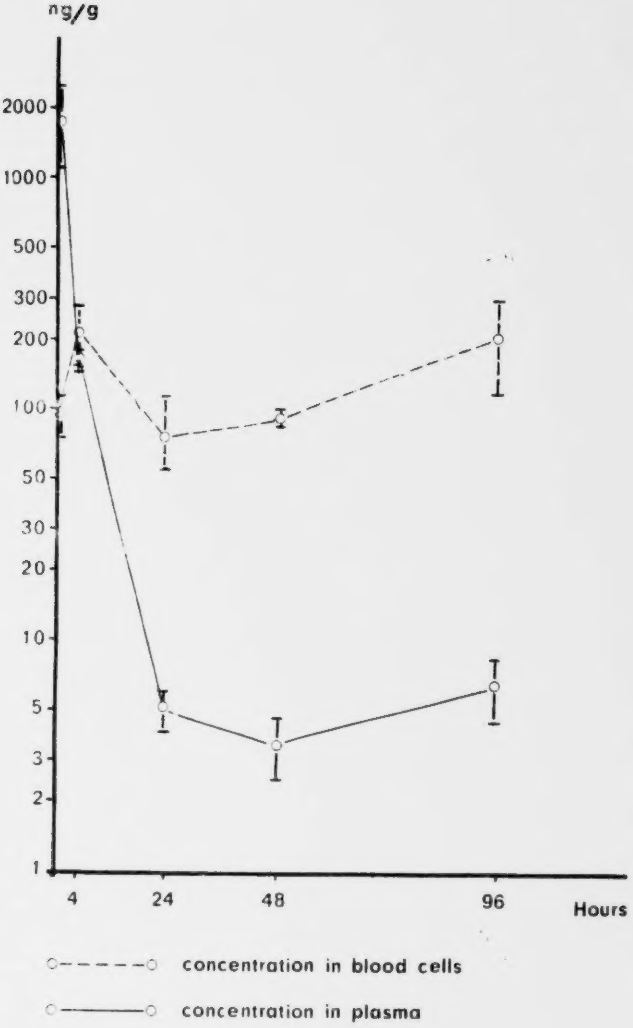


FIGURE 4:1 Concentrations of cadmium in plasma and blood cells respectively in mice given a single subcutaneous injection of $^{109}CdCl_2$ (1 mg Cd/kg) and killed various times after injection. Vertical bars indicate ranges and circles mean values (From Nordberg, 1972a).

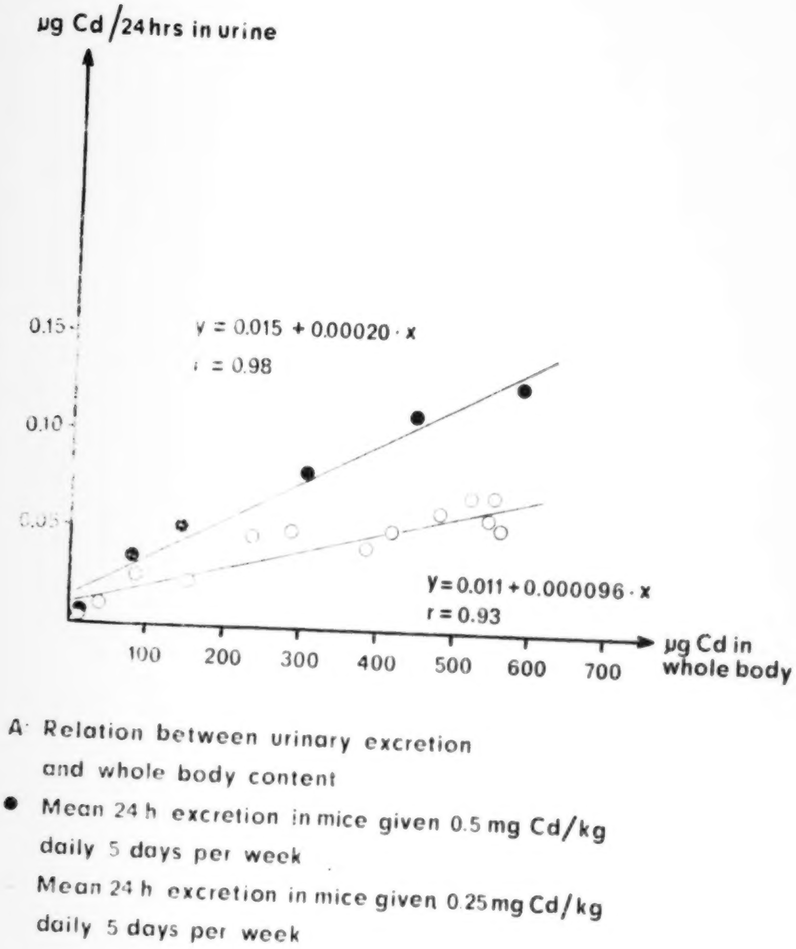


FIGURE 4:2 Relation between urinary excretion and whole body content of cadmium in mice given daily injections of ^{109}Cd (0.25 or 0.5 mg Cd/kg) and followed by whole body and excretion measurements (From Nordberg, 1972a).

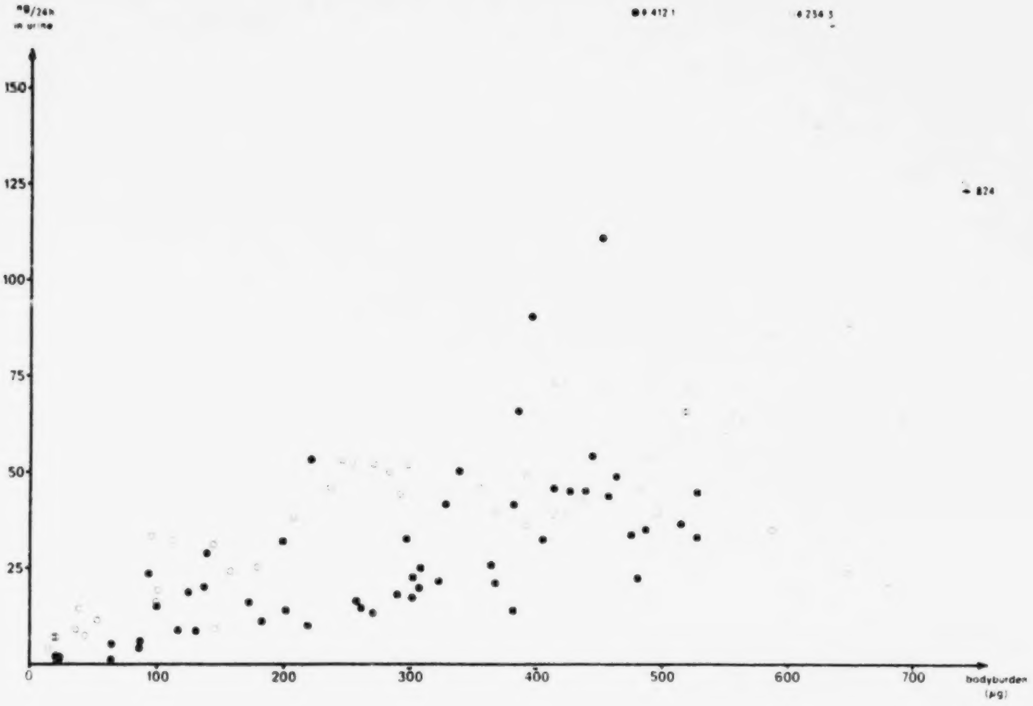


FIGURE 4:3 Individual values on urinary excretion of cadmium related to body burden. All mice were given s.c. injections 0.25 mg Cd/kg body weight per day. (5 days per week).

● = one group
○ = another group, treated in the same way.

(From Nordberg, 1972b and unpublished data).

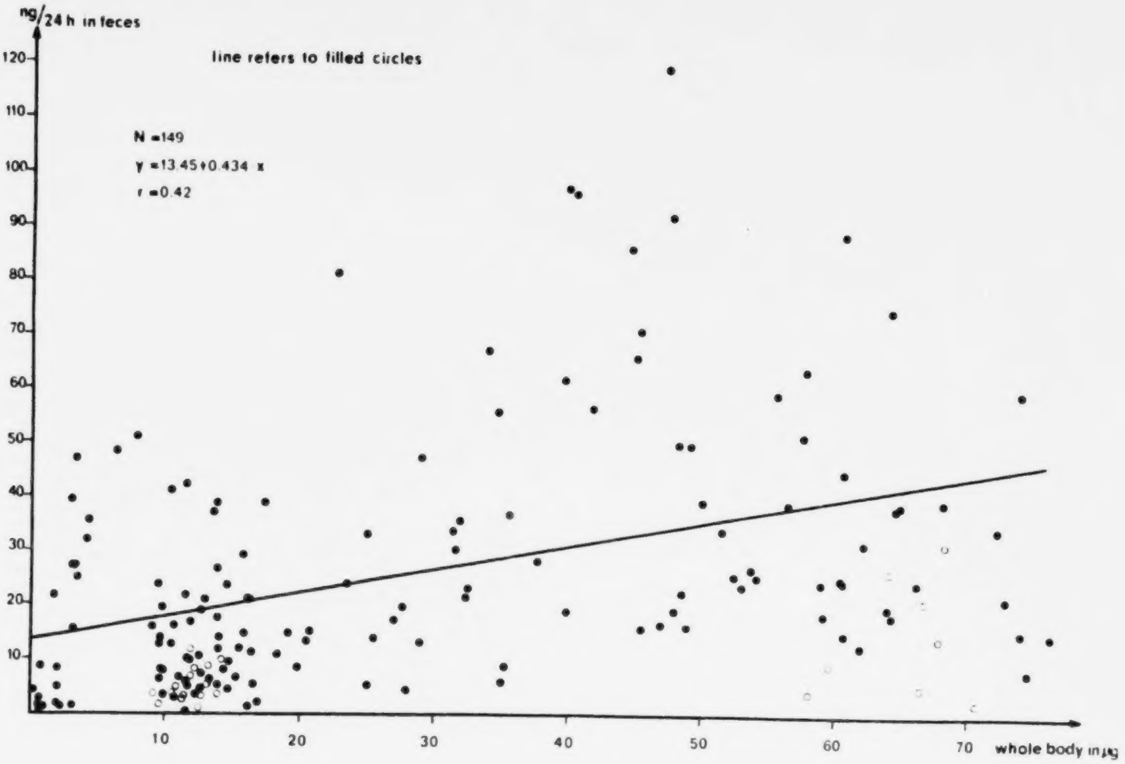


FIGURE 4:4 Individual values on fecal excretion of cadmium related to body burden. All mice were given s.c. injections 0.025 mg Cd/kg body weight per day (5 days per week).

● = during or immediately after exposure

○ = 3 weeks after exposure

(From Nordberg, 1972b).

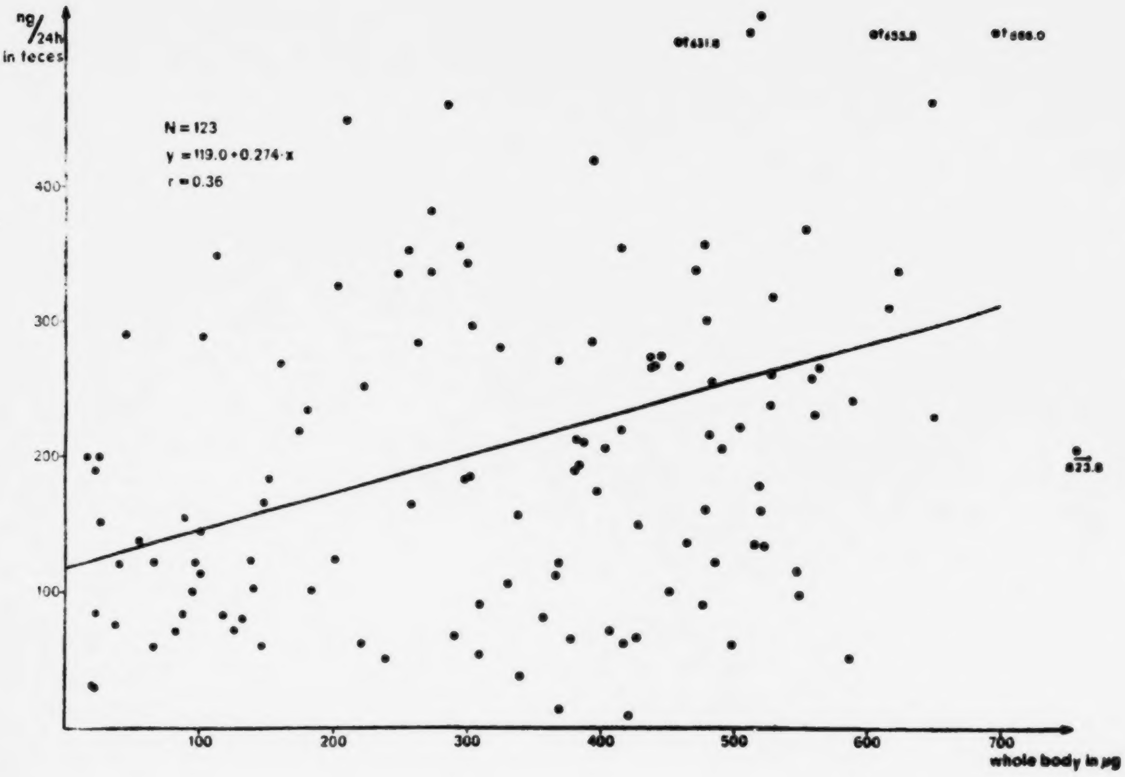
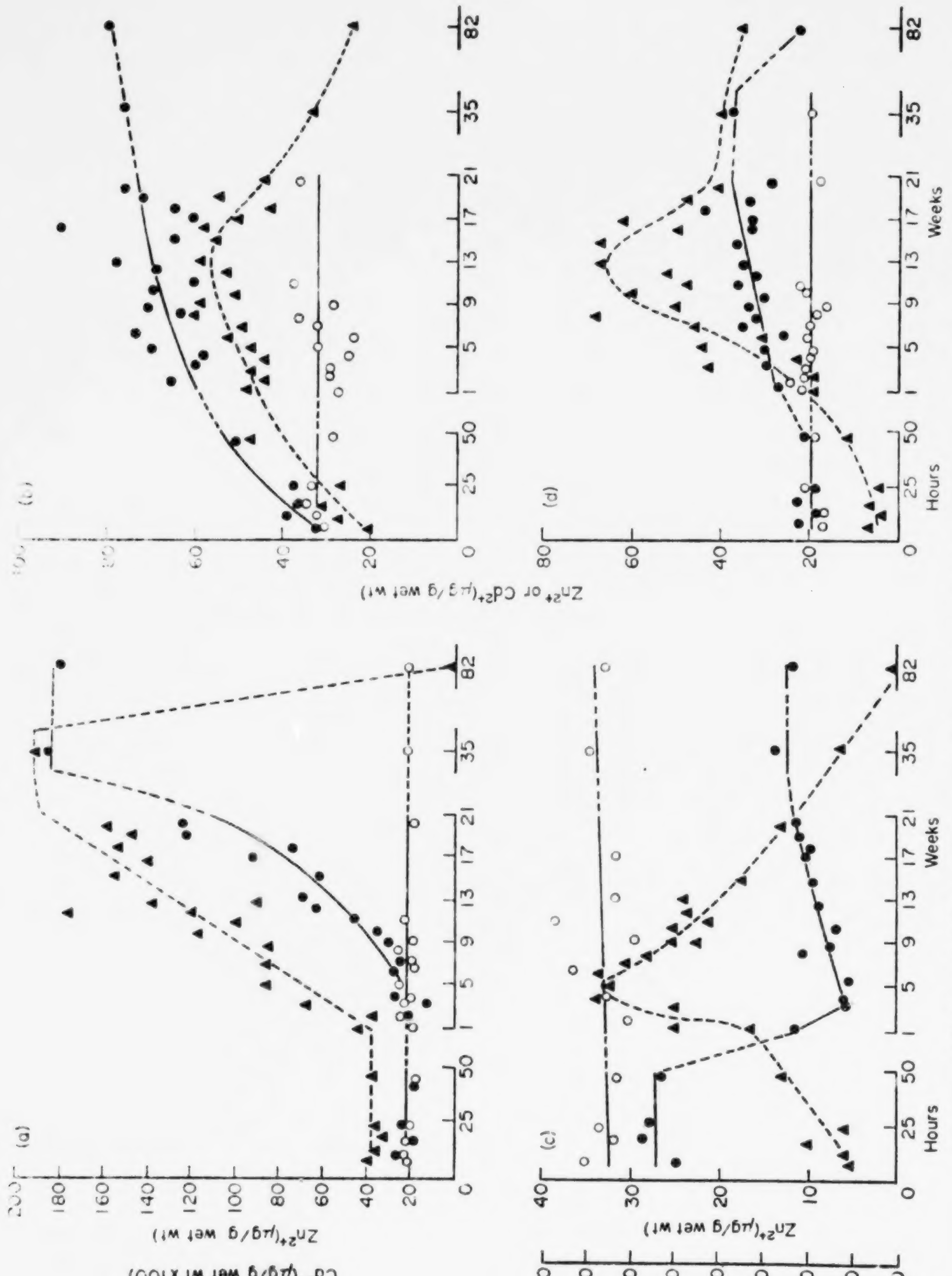


FIGURE 4:5 Individual values on fecal excretion of cadmium related to body burden. All mice were given s.c. injections 0.25 mg Cd/kg body weight per day. (5 days per week). (From Nordberg, 1972b).



Contents of Zn^{2+} and Cd^{2+} in (a) testis, (b) liver, (c) epididymis and (d) kidney at various times after the subcutaneous injection of $CdCl_2$ (2·2 μmol/100 g body wt), into adult male rats. Details of the analytical procedures are given in the Legend to Text-fig. 4 and the 'Materials and Methods' section. Cd^{2+}-injected animals: ▲, Cd^{2+} content; ●, Zn^{2+} content. Control animals: ○, Zn^{2+} content.

FIGURE 4:6 Cadmium and zinc concentrations in rat organs after s.c. injections of $CdCl_2$ (From Webb, 1972a).

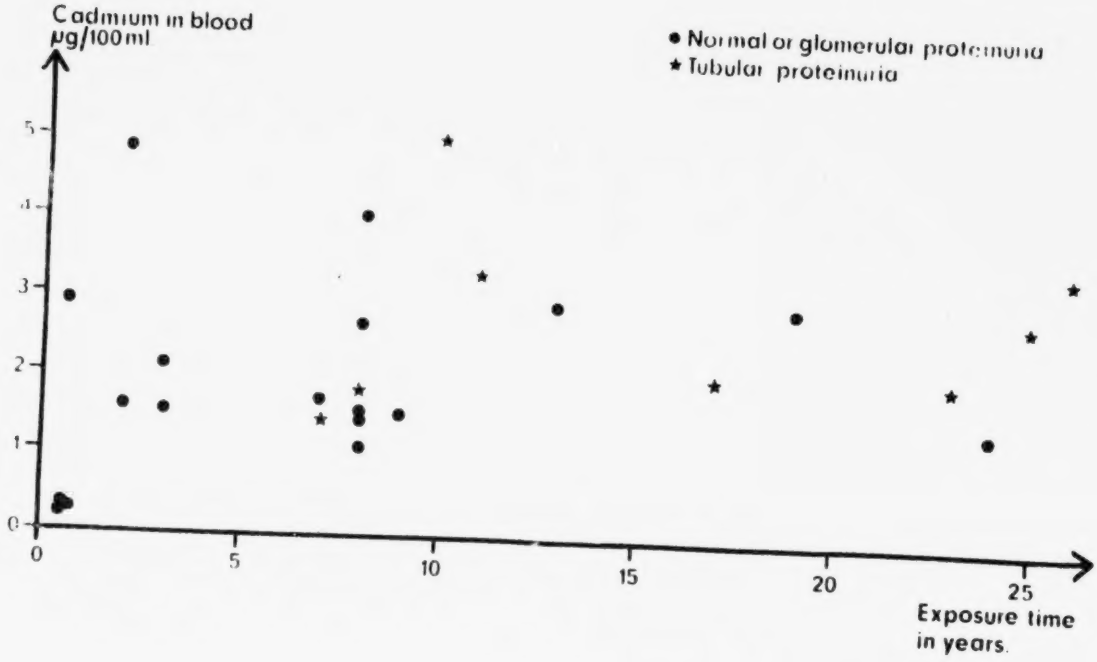


FIGURE 4:7 Relationship between exposure time and cadmium concentrations in blood among workers in an alkaline battery factory (From Piscator, 1972).

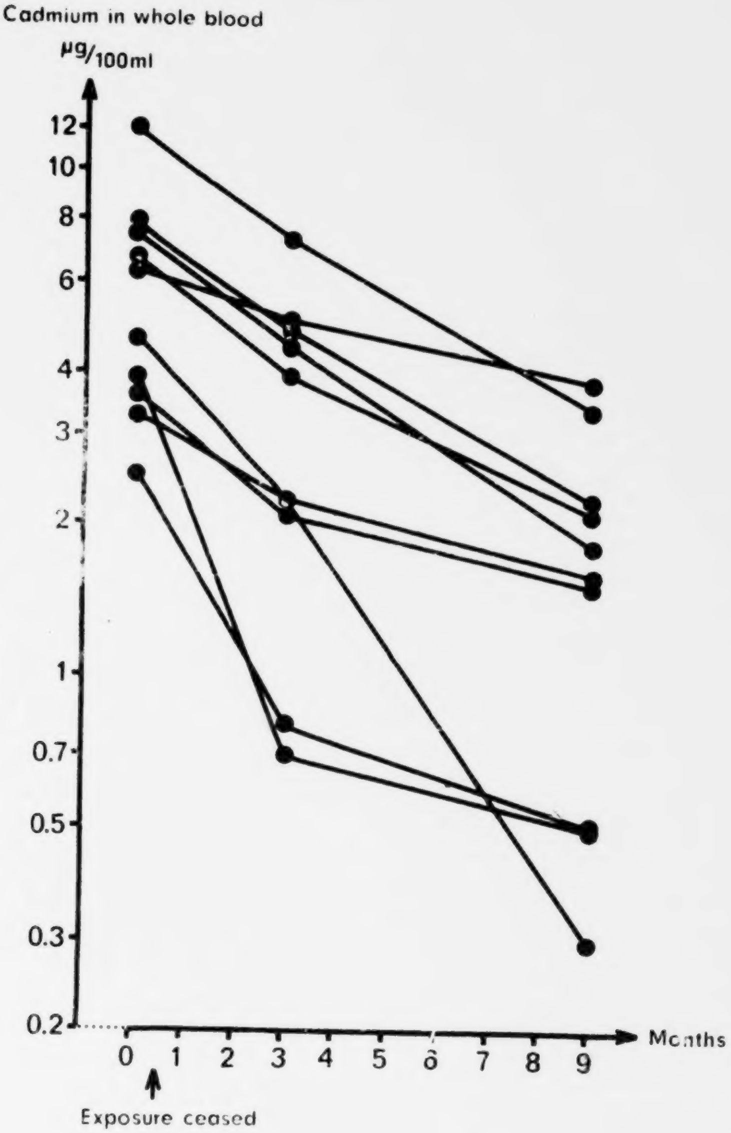
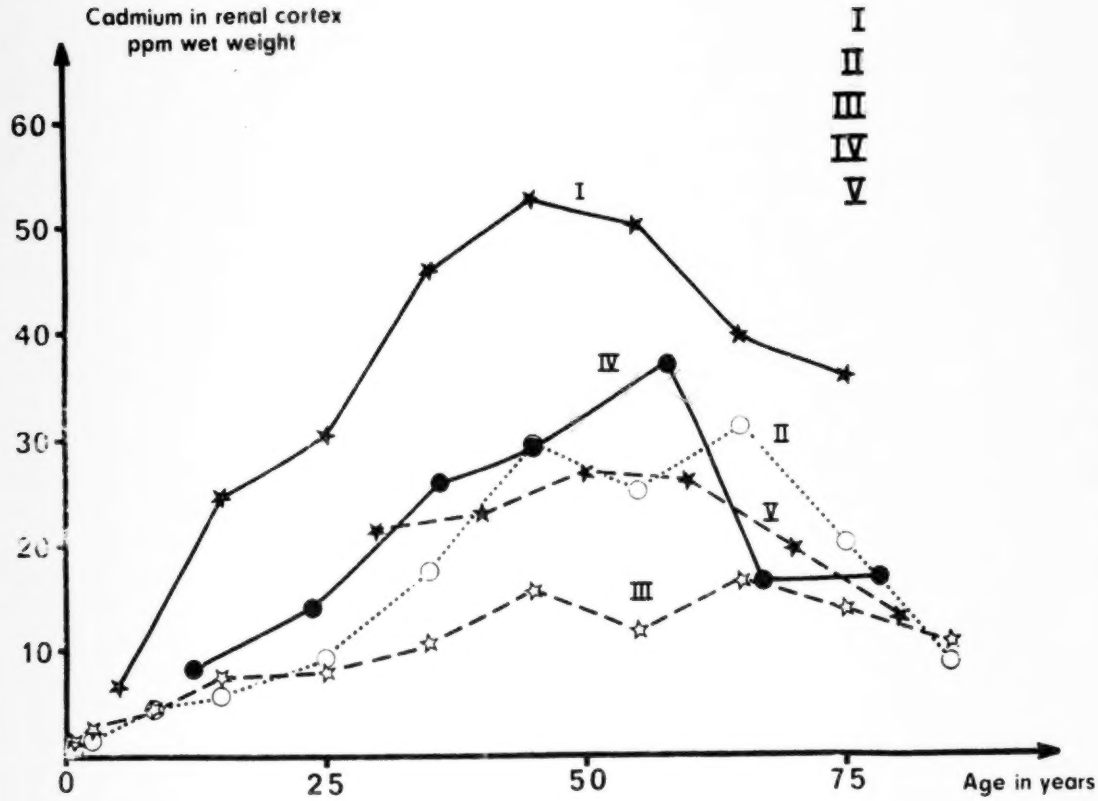


FIGURE 4:8 Cadmium concentrations in blood in workers during and after exposure (Piscator, unpublished data).



I = USA. (From Schroeder and Balassa, 1961).

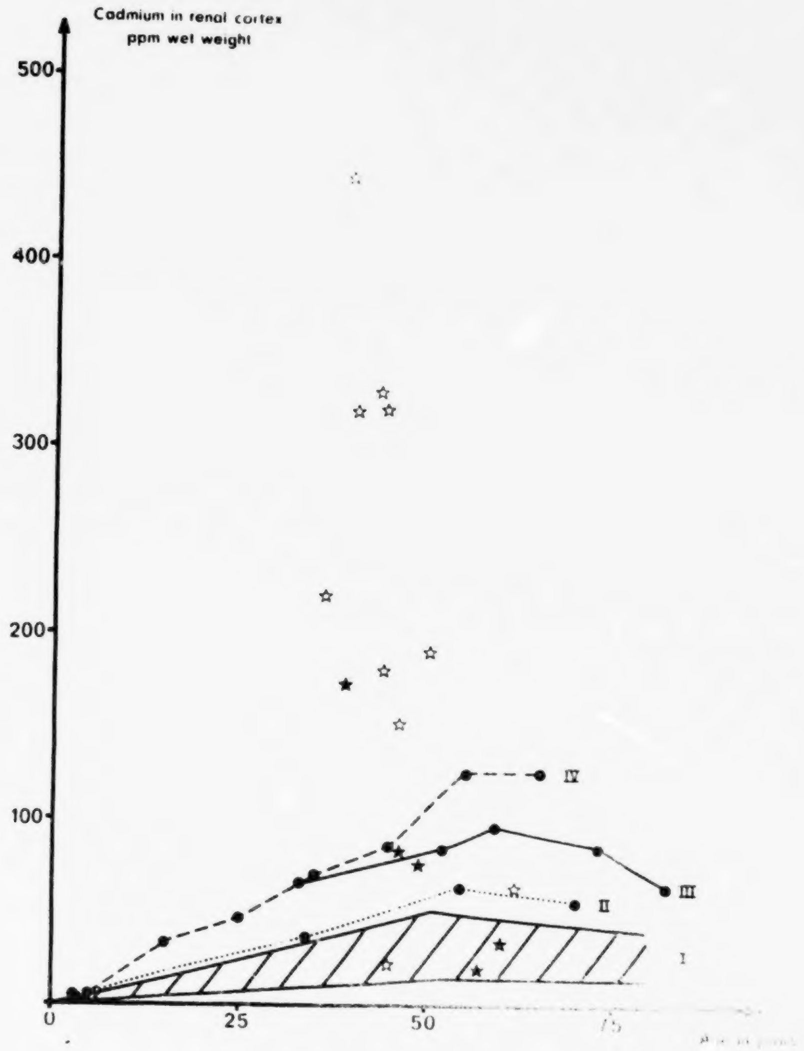
II = Men, East Germany (From Anke and Schneider, 1971).

III = Women, East Germany (From Anke and Schneider, 1971).

IV = Men, Sweden (From Piscator, unpublished data).

V = USA. (From Hammer and Finklea, 1972).

FIGURE 4:9 Cadmium concentrations in renal cortex from normal human beings in different age groups (mean values).



☆ = workers without kidney morphological changes.
★ = workers with " " " .
I = range, USA, Europe (see Figure 4:9).
II = Kobe (From Kitamura, Sumino and Kamatani; 1970).
III = Kanazawa (From Ishizaki, Fukushima and Sakamoto, 1970).
IV = Tokyo (From Tsuchiya, Seki and Sugita, 1972).

FIGURE 4:10 Cadmium concentrations in renal cortex from normal human beings in different age groups (mean values) and exposed workers (single values).

57-m

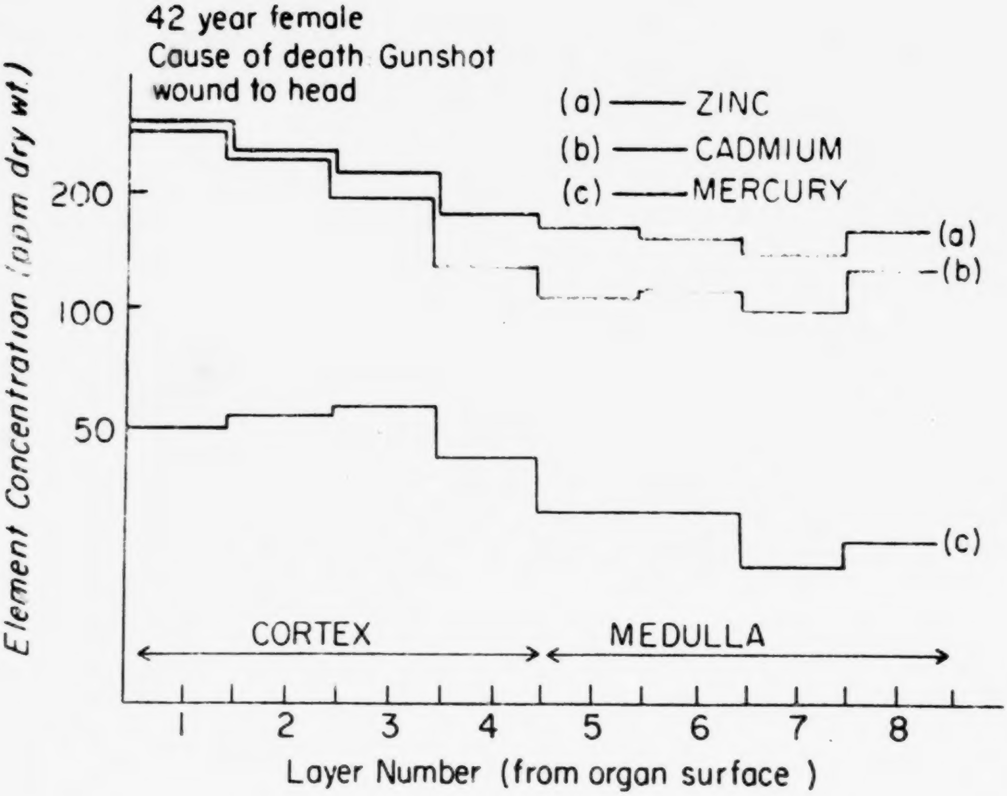


FIGURE 4:11 Cadmium, zinc and mercury concentrations in different layers of the kidney cortex and medulla (From Livingstone, 1972).

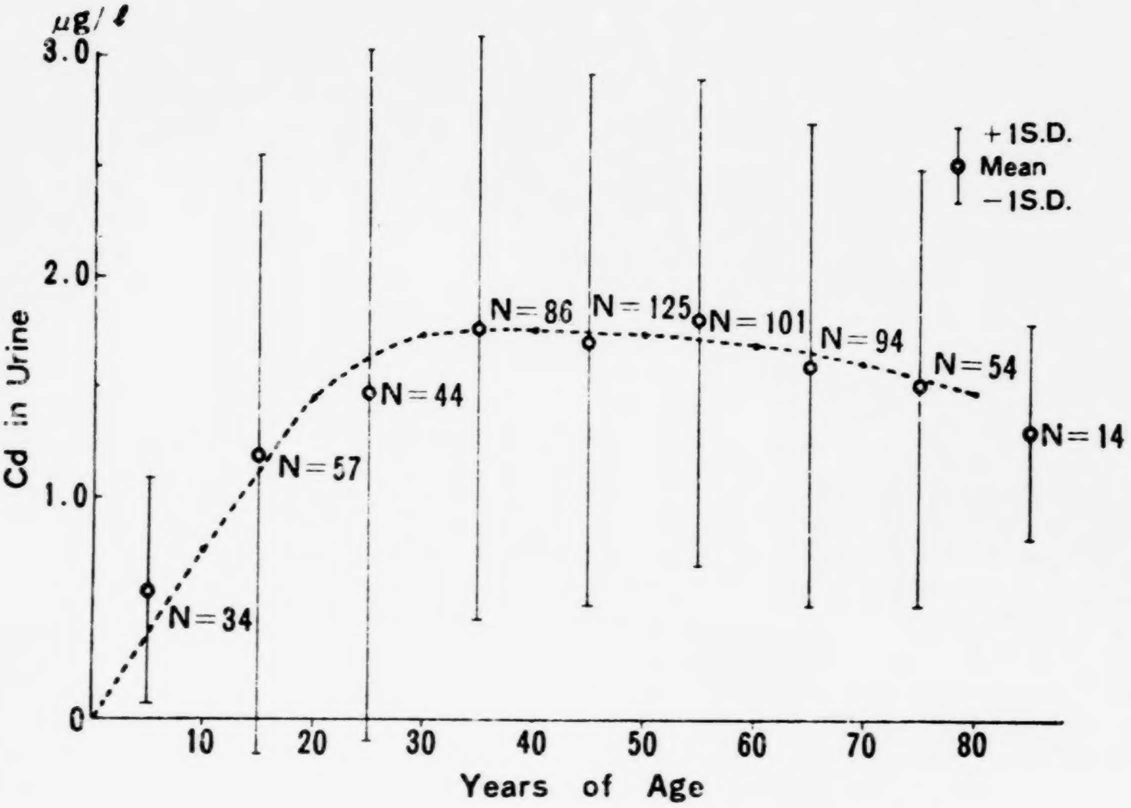


FIGURE 4:12 Average cadmium concentration in urine within different age groups in Tokyo (From Tsuchiya, Seki and Sugita, 1972a).

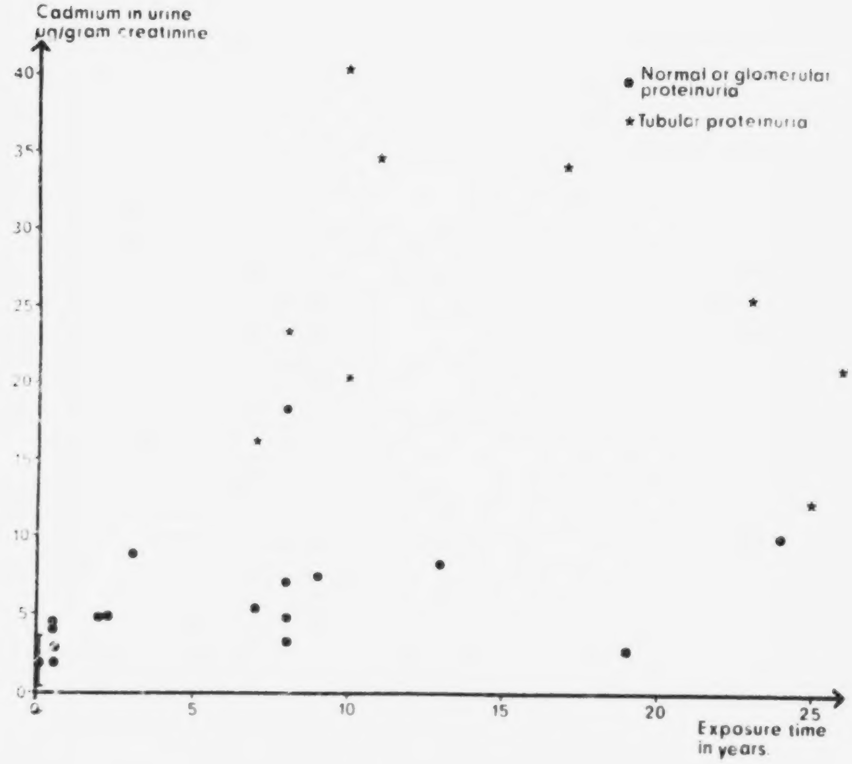


FIGURE 4:13 Relationship between exposure time and cadmium excretion in urine among workers in an alkaline battery factory (From Piscator, 1972).

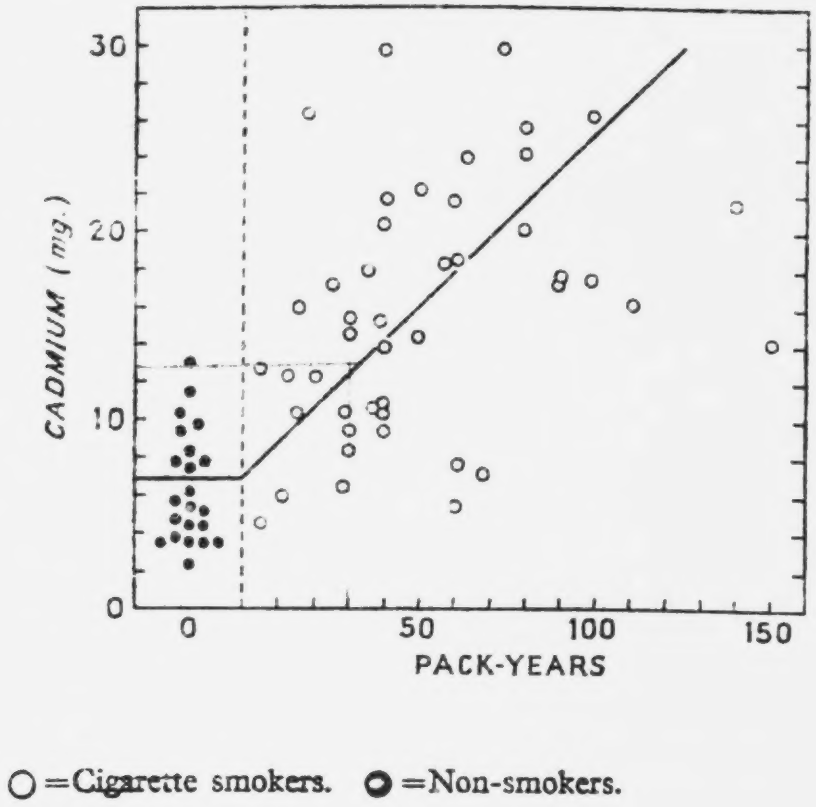


FIGURE 4:14 Cadmium "body burden" (as expressed by the calculated sum of renal, liver and lung burdens) in relation to cigarette smoking (From Lewis et al., 1972).

CHAPTER 5 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Just as in the previous review (CITE) the chronic effects on the kidneys will be given the most extensive treatment here, since the kidney is the critical organ and renal tubular dysfunction is the main feature in chronic cadmium poisoning. Effects which may be related to renal damage, such as effects on bone, will also be discussed. Other effects, resulting from both acute and chronic exposure, were dealt with thoroughly in CITE and will be taken up more summarily here.

Epidemiological studies on proteinuria associated with cadmium exposure in Japan will be referred to in Chapter 6.

5.5 RENAL EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

It is well documented that long-term exposure to cadmium may give rise to renal damage in both animals and man. A characteristic symptom is proteinuria of the tubular type. Cadmium is considered to be transported to the tubules with a low molecular weight protein, metallothionein and has been found to accumulate there with a very long biological halftime. When a level of about 200 ppm is reached in renal cortex, the first sign of tubular dysfunction (tubular proteinuria) may appear in sensitive persons. Furthermore, exposure to cadmium may lead to generalized renal tubular damage with increased excretion of amino acids, glucose, phosphorus and calcium. This may eventually cause changes in metabolism with decalcification and osteomalacia.

5.1.1 In human beings

During the last years only a few new reports have appeared. Goyer et al., 1972, studied the excretion of amino acids in 10 workers exposed to cadmium oxide fume in a Japanese factory producing silver-cadmium alloys. Excretion of cadmium ranged from 7-362 µg/l, meaning that the exposure must have been heavy in many cases. No significant differences in the excretion of individual amino acids were seen when the workers were compared to a control group. Specific increases in the excretion of certain amino acids as reported by Clarkson and Kench, 1956, were not found.

Piscator, 1972, reported on 27 workers exposed to cadmium oxide dust for 0.5-25 years and generally without proteinuria as judged by the results of qualitative tests (Trichloracetic acid). (For details see section 4.3.4.1). It was found that significant increases in the excretion of β_2-microglobulin and changes in electrophoretic patterns could occur without demonstrable increases in total urinary protein. It was also shown that cadmium excretion generally was higher in workers with tubular proteinuria. Nomiyama, 1971b, also found a higher cadmium excretion in workers with proteinuria than in those without it (Trichloracetic acid or Sulfosalicylic acid test).

5.1.2 In animals

Several long-term studies have been reported which give further information on the mechanism for development of renal damage and its relationship to the critical levels in the kidney. Nordberg and Piscator, 1972, found a decrease in the excretion of total protein in male mice given subcutâneous injections of cadmium chloride (0.25 or 0.5 mg Cd/kg body weight 5 days per week for 6 months). The main urinary protein in male mice is a low molecular weight protein, synthesized in the liver, and this synthesis is stimulated by testosterone. A possible cadmium influence on testosterone synthesis could explain a decrease in the synthesis rate of that protein, and in turn, a decrease in total urinary protein excretion. After 21 weeks of exposure, cadmium excretion increased in mice given 0.5

mg/kg. At that time there were also changes in the urinary protein electrophoretic pattern, which earlier had been dominated by the above mentioned testosterone dependent protein. Other proteins appeared which had a pattern indicating renal damage. Further examinations (Nordberg, unpublished data) at the end of the experiment, i.e. after 24 weeks of exposure, showed average concentrations of 170 ppm of cadmium in whole kidney (Table 4:1) in both exposure groups, which corresponds to renal cortex levels of about 170-250 (section 4.2.2).

Piscator and Larsson, 1972, gave cadmium in drinking water (0-10 ppm) to groups of female rats on normal and low calcium intake, respectively. The animals were killed after one year. In rats on calcium deficient diet and drinking water with 10 ppm cadmium, the cadmium level in renal cortex was about 90 ppm. The total protein excretion was not significantly increased in these rats compared to control rats on a calcium deficient diet, but without exposure to cadmium. There was a highly significant increase in ribonuclease, which may be in accord with findings in humans with chronic cadmium poisoning. It may well be that the excretion of ribonuclease in the rats was a very early sign of renal tubular dysfunction.

Nishizumi, 1972, gave male rats cadmium in drinking water at concentrations of 10, 50, and 300 ppm. Animals were killed after 6, 12, 24 and 40 weeks and the kidneys examined by electron microscopy. In rats given water containing 10 ppm cadmium, minor changes were noticed already after 6 to 12 weeks. After 40 weeks slight but obvious changes were seen, such as swollen mitochondria and vacuoles containing cell debris. At high dose levels obvious changes were noted already after 6 to 12 weeks and pronounced mitochondrial changes after 24 to 40 weeks. Cadmium concentrations in organs were not determined.

Berry, 1972, gave male rats subcutaneous injections of 1.4 mg of cadmium sulfate 3 times a week for 5 months. At this high dose level (2-3 mg Cd/kg body weight) proteinuria was said to have appeared after one month (method not stated). Electron microscopy revealed glomerular and tubular lesions after one month. No determinations of the cadmium content of the cortex were performed. At the doses given, very severe renal lesions would be expected.

Ogawa et al., 1972b, reported on an experiment in which mice were given water containing 146 ppm of cadmium. After three months a considerable reduction in carbonic anhydrase activity in blood, liver and kidney was observed.

Stowe, Wilson and Goyer, 1972, gave 10 rabbits cadmium in drinking water (160 ppm) for 6 months. The mean exposure was 15.5 mg Cd/kg body weight per day. Assuming that 1-2 percent had been absorbed, this dose would result in a mean daily absorption of 0.16-0.32 mg/kg. Cadmium was determined in whole kidney and histopathological examinations were carried out by light and electron microscopy. The mean renal concentration was 170 (S.D. 14) ppm wet weight. Calculating with a ratio of 1.5 between cortex:whole kidney (section 4.2.2) would give about 250 ppm wet weight in the cortex. Extensive interstitial fibrosis was seen by light microscopy in the kidneys of exposed animals. Pronounced changes were seen in the proximal tubules, including pyknosis, karyorrhexis and epithelial sloughing. Electron microscopy also showed seemingly unaffected mitochondria. Collagen was deposited in the glomeruli. Amyloid deposits could not be demonstrated.

Castano and Vigliani, 1972, have studied the fate of horseradish peroxidase (a low molecular weight protein) when intravenously injected into cadmium exposed rats with weights of about 300 g.

Cadmium (0.16 mg Cd, 3 times a week for 1-4 weeks) was given intraperitoneally as cadmium chloride. The results indicated that horseradish peroxidase, after being filtered through the glomerular membrane, was reabsorbed in a normal way. Electron microscopy revealed normal pinocytosis. No determinations of cadmium in the kidneys were performed. An estimate of the concentration of cadmium in renal cortex can be obtained by comparison with an experiment by Bonnell, Ross and King, 1960, who gave rats intraperitoneal injections 3 times a week in doses of 0.75 mg/kg. This dose resulted in an average renal concentration of about 60 ppm (cortex levels not above 100 ppm wet weight) after one month. Since the dose given by Castano and Vigliani was 2/3 of that given by Bonnell, Ross and King, the renal cortex level could be expected to be below 100 ppm.

Regarding the mechanisms for the accumulation and toxic effects of cadmium in the kidney, a study by Nordberg, 1971a, is of interest. He gave mice subcutaneously a single dose of cadnium (1.1 mg/kg) together with metallothionein. Renal tubular damage resulted while no such effect was seen in another group of mice given a corresponding dose of cadmium as $CdCl_2$ alone. It is conceivable that cadmium bound to metallothionein passed the glomeruli and was reabsorbed into the tubules. (This experiment is also described in section 4.5). These acute effects are similar to those described after injection of cadmium together with cysteine (Gunn, Gould and Anderson, 1968b) or together with BAL (Gilman et al., 1946, Dalhamn and Friberg, 1955) or EDTA (Friberg, 1956).

5.1.3 Dose-response relationships

In the previous review it was estimated that 200 ppm wet weight is the critical level in the renal cortex. This is the level at which the first signs of tubular dysfunction may appear in sensitive individuals. This estimate was based mainly on data on kidney concentrations of cadmium, determined after death or after renal biopsy, under most circumstances from workers exposed to cadmium. In cases with no or slight proteinuria, and without histological changes, renal cortex levels were around 200 ppm wet weight or higher. In cases with long standing proteinuria and morphological renal damage, renal cortex concentrations were low, often below 100 ppm wet weight. This was explained by the fact that whenever renal damage becomes apparent, large amounts of cadmium are lost, probably due both to decreased reabsorption of cadmium containing proteins, and to losses of renal cells. From animal data, which supported the 200 ppm estimate, and available data on absorption and excretion rates in normal human beings, the doses necessary for reaching the critical level under different conditions were calculated.

During the last years some new data have been obtained which have given further information about levels at which renal dysfunction may occur. Nomiyama, 1971b, describes the findings on autopsy of one woman and two men (all 3 without occupational exposure) from the Annaka district where a large zinc smelter is situated. He also had examined these people prior to their deaths and the comparative results are seen in Table 5:1. Nomiyama is of the opinion that the critical level in the renal cortex cannot be as low as 200 ppm, stating that in one case he found a level of 260 ppm without renal damage. The term critical level, as used in the present report, however, means the level at which sensitive persons may suffer renal tubular dysfunction. This implies that in a population one may find many persons with renal cortex levels above 200 ppm but without renal tubular dysfunction. Moreover in the case described by Nomiyama, no electrophoretic examination of urinary proteins had been carried out. Slight tubular dysfunction as characterized by pathological electrophoretic patterns but with negative qualitative proteinuria tests may well exist without any morphological changes in the kidneys.

Another of the descriptions by Nomiyama involved a man aged 45 who had lived in the same area all his life. In this case glucosuria and tubular proteinuria were present. The cadmium concentration in renal cortex was very low, only about 24 ppm, while the concentration in pancreas was very high, about 20 times higher than normal. This case fits with earlier observation of very low cadmium concentrations in renal cortex synchronous with severe renal damage despite high concentrations in liver and pancreas.

The studies by Nordberg, 1971a, (see sections 4.2.2 and 5.1.2) give additional evidence that the critical level is about 200 ppm. In one group of mice given 0.25 mg Cd/kg body weight, 5 days a week, renal tubular damage was not found after 24 weeks of exposure, whereas in a group given 0.5 mg Cd/kg body weight, 5 days a week, renal tubular dysfunction was apparent after 21 weeks of exposure. Both groups were killed 24 weeks after the beginning of the exposure and in both groups renal levels of cadmium were about 170 ppm, corresponding to cortex levels of 170-260 ppm wet weight. Liver levels were about the same in both groups, whereas levels in other organs (Figure 4:1) were about twice as high in the group which had received the higher concentration of cadmium. It may be remembered that the study by Stowe, Wilson and Goyer, 1972, also supported a critical level of about 200 ppm in kidney cortex.

As has been mentioned earlier in this chapter, Piscator and Larsson, 1972, studied the excretion of ribonuclease in rats exposed to cadmium in drinking water. In rats on a calcium deficient diet, an increased excretion of ribonuclease was found at renal cortex levels of about 90 ppm, indicating a considerably lower critical level than the one estimated in earlier investigations covered in CITE. Estimates of critical level were based on results obtained by relatively crude methods and it was suggested that cellular effects may well appear at lower levels. Furthermore, the rats in Piscator and Larsson's studies were severely deficient in calcium, a condition not uncommon in large populations in several areas of the world.

5.2 EFFECTS ON BONE

In CITE it was shown that exposure to cadmium in calcium deficient rats would cause a rapid demineralization (Larsson and Piscator, 1971). Further analysis of the data showed that calcium deficiency gave rise to a compensatory increase in the parathyroid volume. If the animals were exposed to cadmium, such an increase in parathyroid volumes did not occur. New experiments (Piscator and Larsson, 1972) revealed an increase in excretion of ribonuclease in female rats after one year of exposure to 10 ppm of cadmium in drinking water and a diet low in calcium. This finding indicates renal tubular damage. There was also a significant reduction in the mineral content of bone. Whether the bone changes were due to the changes in renal function or due to an effect of cadmium on intestinal absorption of calcium is not known.

More evidence that exposure to cadmium may cause bone changes in animals comes from Hirota, 1971. He exposed rabbits for about one year to cadmium in water corresponding to daily doses of 5 and 20 mg Cd per rabbit. The rabbits exposed to 20 mg Cd/day showed histological signs of renal tubular changes at the end of the exposure. X-rays of femur caused him to suspect osteomalacia and osteoporosis. An increase in serum calcium and decrease in serum phosphorus as well as an increase in the urinary excretion of calcium and phosphorus were also seen. Proteinuria was noted in some animals.

5.3 EFFECTS ON THE LIVER

In CITE it was concluded that liver dysfunction is not common in workers exposed to cadmium. Animal experiments indicate that certain enzyme activities may be influenced at cadmium levels in the range found in normal human adults.

A recent investigation on rabbits by Stowe, Wilson and Goyer, 1972, gives additional information. The experimental conditions have been described in sections 4.2.2 and 5.1.2. After 6 months of oral exposure to cadmium, mean liver concentrations were 188 ppm wet weight. Light microscopy revealed that in contrast to controls the cadmium exposed rabbits showed depletion of glycogen and deposits of collagen. Inflammatory cell infiltrates were frequent in the portal regions and biliary hyperplasia was often present. Electron microscopy revealed that the most striking changes took place in the endoplasmic reticulum, which was increased in exposed animals.

Liver function tests, such as determination of activity of GOT, GPT, alkaline phosphatase and LDH isoenzymes in serum, BSP-test and blood coagulation tests did not show any difference between controls and exposed. These results indicate that negative liver function tests do not exclude morphological liver changes.

5.4 OTHER EFFECTS

Pond and Walker, 1972, showed that a single prophylactic injection of iron can prevent the occurrence of cadmium anemia in growing rats exposed for 4 weeks to 100 ppm of cadmium in the diet. Addition of iron to the food had the same preventive effect. Fox et al., 1971, studied Japanese quail fed a diet containing 75 ppm of cadmium. In addition to iron, ascorbic acid prevented anemia. According to the authors ascorbic acid was a good prophylactic because it brought about increased absorption of iron from the intestinal tract. Weber and Reid, 1971, reported that ascorbic acid partially reduced the toxicity of cadmium in chicks.

Schroeder and Mitchener, 1971, looked for teratogenic and reproductory effects of cadmium. Mice were exposed during two generations to cadmium in drinking water (10 ppm) from the end of the weaning period until the end of the experiment. Cadmium was found toxic for breeding mice to such an extent that the strain did not survive beyond the second generation. Congenital abnormalities appeared at a much higher frequency than in controls. Cadmium and zinc were not determined in organs. Since cadmium does not transverse the placenta, it is conceivable that a teratogenic effect would be due to a secondary zinc deficiency in the fetuses. The mothers retain more zinc during exposure to cadmium, leaving less zinc available for the fetuses. During exposure to cadmium, more zinc than normal is stored in liver and kidneys and less in testes (Petering, Johnson and Stemmer, 1971). The only data available on humans in this context are those already taken up in CITE, showing, in short, a significantly decreased weight in newborns of cadmium exposed women (Cvetkova, 1970).

Biochemical effects of cadmium, especially with regard to effects on enzymes, have recently been treated in an extensive review by Vallee and Ulmer, 1972.

5.5 CONCLUSIONS

Animal studies have verified that cadmium exposure may cause renal tubular damage. This damage occurs at cadmium levels of about 200 ppm wet weight in renal cortex, which is in accordance with the earlier estimate of 200 ppm as the critical level in the cortex. In calcium-deficient animals exposed to cadmium, urine studies indicate that this level may be even lower under certain circumstances.

Investigations on animals and on workers exposed to cadmium have further strengthened the earlier statement that the excretion of cadmium will increase when tubular dysfunction appears. Severe morphological changes may be found in the liver of animals even though common clinical tests for liver function are normal.

Further studies of the effects of cadmium on bone have shown that in animals cadmium exposure may cause demineralization. In calcium-deficient animals this process will be accelerated by cadmium. An influence on the parathyroids has been found.

TABLE 5:1 Results of examination before and after death of three persons from Annaka. (From data by Nomiyama, 1971b).

Case designation number	1	2	3
Age	45	57	84
Sex	M	M	F
Estimated intake of cadmium	211 (1969)	350 (1969)	Not known
Liver Cd, ppm wet weight	35	32	29
Renal cortex Cd, ppm wet weight	29	264	134
Pancreas Cd, ppm wet weight	41	24	18
Cd µg/l urine	5-24	6-25	N.D.
Urine protein	+[x]	-	N.D.
Glucose	+	N.D.	N.D.
Morphological changes	+	-[xx]	+

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[x] Examination by electrophoresis and gel filtration revealed that the proteinuria was of a tubular type.

[xx] In the report it is stated "only such a change as tangle of renal tubule cells was observed".

N.D. = Not detectable

CHAPTER 6 EPIDEMIOLOGICAL INVESTIGATIONS IN CADMIUM-POLLUTED AREAS OF JAPAN

6.1 INTRODUCTION

In our previous review Cadmium in the Environment (CITE) it was seen that the prevalence of proteinuria and glucosuria in older age groups was higher in the cadmium polluted Itai-itai disease district, Fuchu, in Toyama Prefecture, than in an unpolluted non-endemic district (Figure 6:1). This difference in prevalences was concluded to be related to cadmium exposure. Another cadmium polluted district, which was presented in CITE and compared to a control area was a part of Tsushima in Nagasaki Prefecture. Proteinuria among older age groups was more common in the polluted than in the control area (Figure 6:2).

Other areas of Japan were under study for the effects of cadmium pollution. Based on cadmium concentrations in rice and soybean paste ("miso") it could be ascertained that in some of these other areas cadmium exposure was considerably higher than in control areas. As cadmium is used extensively in mining, smelting and other industrial operations throughout Japan, it was inferred that the cadmium problem may well be far-reaching.

In order to map the extent of possible effects of cadmium the Japanese Ministry of Health and Welfare and the authorities of the prefectures concerned have during the last years made a number of epidemiological studies in at least 13 areas suspected for cadmium pollution.

Data on daily cadmium intake are scarce. However, cadmium concentrations in rice are usually available due to the fact that the Ministry of Health and Welfare has ordained that in the areas suspected for cadmium pollution yearly analysis of cadmium in rice should be undertaken.

Past changes in daily intake are important for evaluating possible effects (Kjellström and Friberg, 1972) but as there are generally no data on past cadmium concentrations in rice, possible changes often have had to be inferred from production changes at the cadmium source.

Most of the data on medical effects presented below concern proteinuria. Of all possible indicators of an effect, proteinuria is the only one which has been measured and reported on consistently, though the methods for analyzing it vary.

Available data which have been considered of interest for discussing dose-response relationships have been included. The data have been collected through personal visits to Japan, from published articles and from a large number of internal mimeographed reports and unpublished data. In Figure 6:3 the places which will be mentioned in this report can be found.

6.2 METHODOLOGY

Much of the medical data used in this report have resulted from health screenings in the polluted areas. These have generally been performed according to standard methods recommended by the Ministry of Health and Welfare and these methods will be described briefly in this section. Methods for estimating daily intake are also presented and discussed.

6.2.1 Method for selection of areas studied for cadmium-pollution

The Ministry of Health and Welfare, 1969, published a standard method for selection of "areas requiring observation for cadmium-pollution". If within a village values of cadmium concentration in drinking or irrigation water higher than 0.01 ppm or values in unpolished rice higher than 0.4 ppm are found, urine samples from 30 randomly selected adult persons from the village should be collected. The samples are mixed and if the cadmium concentration in this mixed sample is more than 9 µg/l, or if the average daily cadmium intake in the village can be calculated to be more than 300 µg, the village should be designated as an "area requiring observation". In most polluted districts a large number of villages is included in the "area requiring observation".

It should be pointed out that in some of the areas discussed in section 6.3 (e.g. Annaka) cadmium concentrations in rice over 0.4 ppm have been recorded outside the "polluted area". In large parts of the "polluted area" in Bandai cadmium concentrations in rice do not exceed 0.4 ppm, according to recorded values.

6.2.2 Standard methods for screening of cadmium-related disease

According to the Ministry of Health and Welfare, 1972 two types of health screening have been used. The old type of health screening (used until 1971) was divided into two principal stages. The first stage was a general screening of the population over 30 years of age in defined areas. Included in the first screening were: Interview, proteinuria analysis (Trichloracetic acid), glucosuria analysis (testtape) and blood pressure measurement.

All persons who were confirmed as having proteinuria according to the first screening should continue to the second screening. This rule of the standard method has not been strictly kept in all areas, which is obvious when going through the listings of the individual results (Japanese Association of Public Health, 1970a). The difference between the number with proteinuria and the number studied in the second screening is less than 10 percent in all cases that have been possible to check.

In the second screening a more detailed study on blood and urine was performed and the results of this study were used for evaluating if a person had any effects of cadmium exposure.

In May, 1971, the Ministry of Health and Welfare published a new standard method for screening cadmium-related disease (Ministry of Health and Welfare, 1971b). The target population should be persons over 30 years of age. For qualitative proteinuria measurements the Trichloracetic acid method or the Sulfosalicylic acid method or both were recommended. In this new standard method cadmium concentration in urine should be measured in the first screening. Glucosuria measurement was moved to the second screening, where also measurement of proteinuria (any quantitative method could be used, but should be clearly described) and cadmium in urine as well as disc electrophoresis of urinary proteins were to be performed.

If the disc electrophoretic pattern showed tubular damage at the second screening a third and final screening should be performed. It included a number of blood and urine tests as well as X-ray of certain bones. A distinction was made between those who had a cadmium concentration in urine over 30 μg/l and those who had under 30 μg/l. This level was referred to in Japanese Association of Public Health, 1970b, as a lower limit for pathological cadmiumuria.

The result of the third screening should be analyzed in the following way: The study group for differential diagnosis of cadmium poisoning and Itai-itai disease set up by the Japanese Association of Public Health discusses the results of all persons who undergo the third screening and a final conclusion is made.

As noted here the screening procedures are aimed at finding suspected cases of Itai-itai disease. As the screenings do not primarily aim at detecting the prevalence of proteinuria, the urine sample collection methods as well as analysis methods and classification methods of proteinuria are not uniform from district to district and in some cases not even within districts.

6.2.3 Standard method for qualitative measurement of proteinuria (Ministry of Health and Welfare, 1971b)

Either one of methods a) or b) may be used. It is preferable to combine them.

a) Trichloracetic acid (TCA) method
To 5 ml urine 5 ml of TCA-solution is added (concentration = 35 g/dl).
Foaming should be avoided when mixing and the mixture is heated to 37°C for 10 minutes.

b) Sulfosalicylic acid (SA) method
3 ml urine samples are put into two test tubes of equal size. In one of the tubes a solution of sulfosalicylic acid (20 g/dl) is added drop-wise. The other tube is used as control for evaluation.

Evaluation: same in both a) and b).
Transparent = - ; Faint white cloudiness = ± ; white cloudiness = + ; Strong white cloudiness = ++ ; milk-colored cloudiness = +++ ; precipitate = ++++ . In the final reports proteinuria has usually only been classified as positive and negative,

in some cases using + or higher as the criterium and in other cases ± or higher. Another drawback of most of the epidemiological studies on proteinuria is that the analyses are not made on a "blind" basis. The persons who made the analyses knew whether the urine sample to be analyzed was from an "exposed" area or a "control" area. When studying proteinuria with methods relying on subjective evaluations, obvious possibilities for systematic errors exist if the studies are not made on a blind basis.

6.2.4 Comparisons of proteinuria methods

A study in which proteinuria methods have been systematically compared is the one by Fukuyama, Shiroishi and Kubota, 1971. They compared the Trichloracetic acid, Sulfosalicylic acid and G-25 gel filtration methods with disc electrophoresis of urine. The studies were made on a blind basis (Kubota, personal communication).

In the G-25 method (Fukuyama and Kubota, 1970), urine samples are eluated (20 ml/h in phosphate buffer) on a Sephadex G-25 column (300 × 9 mm) and 2 ml fractions are collected. Folin-Lowry reagent is added to each fraction and the absorbance at 750 nm is measured. An elution pattern of Folin-Lowry positive substance is achieved and usually three peaks (P, Q, and R) are found, the first of which has been found to consist mainly of proteins (Fukuyama and Kubota, 1970). The G-25 protein quotient is calculated as P/(P+Q+R). The G-25 gel filtration method is rated as + if the G-25 protein quotient is higher than 15 percent, ± if the quotient is 10-14.9 percent and - if the quotient is less than 10 percent (Fukuyama, Shiroishi and Kubota, 1971).

In Table 6:1 results from a polluted area in Toyama and a control area are compared. In the polluted district the prevalences of proteinuria rated as + according to Sulfosalicylic acid method and Trichloracetic acid method, respectively are 12.7% and 7.9% and considerably higher (38.2%) with the G-25 method. On the other hand, in the control district the prevalences of proteinuria according to the Sulfosalicylic acid method, Trichloracetic acid method and G-25 method were 1.3%, 2.2% and 1.3% respectively. Data on spec. grav. are not given, which would have been of value when interpreting the results. The G-25 method is not dependant on spec. grav., whereas the result of acid tests will be dependant on the degree on the concentration of urine. It is conceivable that there are cases with decreased concentrating ability in the polluted area.

Fukuyama, Shiroishi and Kubota, 1971, also studied the urines of the persons from the Itai-itai district with the disc electrophoresis method, where disc rated + means that the pattern of the electrophoresis is of the tubular type. As can be seen in Table 6:2 about one third (30.3%) of those studied had tubular proteinuria. Among those who had a rating of at least ± according to the Sulfosalicylic acid method or the Trichloracetic acid method, or had + in the G-25 method, about 70-80% were tubular proteinurias. Out of the 50 persons with disc +, 47 also were positive with the G-25 method. 25 persons resp. 33 persons were positive when using the Trichloracetic acid method resp. Sulfosalicylic acid method.

Another study in which two proteinuria methods have been compared is the one by Kakinuma et al., 1971. 2436 persons from Annaka were studied with both the Trichloracetic acid method and the Sulfosalicylic acid method. A good correlation between the two methods was found (Table 6:3). One of the methods for calculating prevalence of proteinuria (+ or higher) in an area will give about 10% lower figures than if both methods are used (+ or higher in either of them). When setting the limit for proteinuria at + or higher a combined use of both methods will give 10% higher figures than the Trichloracetic acid method alone and less than 1% higher figures than the Sulfosalicylic acid method alone (Table 6:3).

If in two studies on the same population the evaluation criteria would be so different that all persons in the ± group of one study would be included in the + group of the other study, the recorded prevalence of proteinuria as defined by ratings of + or higher would be about twice as high in the latter study as in the first study.

6.2.5 Methods for estimating daily intake

Cadmium concentration in rice will have to be used as an indicator of daily intake when no other data are available, which is common. In 4 areas National Nutrition Census (Japanese Association of Public Health, 1970b) and Total Diet Collection (Japanese Association of Public Health, 1970b) have been used. Yamagata et al., 1971, have used a Standard Diet method. In some cases an estimation based on rice only (Rice-method) has also been used.

The National Nutrition Census method (Japanese Association of Public Health, 1970b) is based on data on average daily intake of food divided into 22 different groups and the average cadmium concentration in each food group. A questionnaire is sent to a sample of families in the polluted and the control areas. They are instructed to record the amount of each of 22 food groups consumed in the family for three days. It was not defined exactly (Japanese Association of Public Health, 1970b) how the families are to be selected. Using figures on the relative calorie requirements in different age groups the family food intake data were adjusted to an "average adult" in the area. Samples of the different food groups were collected from some families included in the questionnaire survey. The collection procedure was systematized only for stored rice and "miso" soybean paste. Cadmium concentrations in the food samples were analyzed with atomic absorption spectrophotometry. In the control areas samples of only a few food groups were collected and the calculation of daily cadmium intake was mainly based on assumed values.

The Total Diet collection method (Japanese Association of Public Health, 1970b) requires that the persons participating put a "twin" sample of each foodstuffs they eat during one day in a container. The material in the container is homogenized and cadmium concentration is analyzed with atomic absorption spectrophotometry.

The Standard Diet method (Yamagata et al., 1971) bases the daily cadmium intake calculation on a specific standard menu recommended by the National Institute of Nutrition. Samples of seven major food groups included in the menu were collected from the area to be studied and analyzed for cadmium concentration. The daily intake was then calculated by multiplying the standard amount of every food group with the measured cadmium concentration.

The Rice-method is an estimation of daily intake based on the assumption that on the average half the daily cadmium intake comes via rice. In the studies by the Japanese Association of Public Health, 1970b 17-83% of the calculated daily intake came via rice. The daily intake of rice is assumed to be 300 g (Association of Health Statistics, 1972).

The variation in estimated daily intake within an area, as indicated by the range, is a crucial factor when assessing dose-response relationships. The individual intakes in polluted and control areas overlap in Uguisuzawa and Tsushima (Table 6:4) and there is a 2-fold to 10-fold increase in intake between minimum and maximum value. Dose-response analysis based on daily intakes estimated with National Nutrition Census (=NNC) or Total Diet Collection (=TDC) will therefore be very approximative

The quotient between intake in a polluted and corresponding control area differs depending on the method used. In Annaka the quotients according to the NNC resp. TDC methods are 2 resp. 3 (Table 6:4) but cadmium concentration in rice in the control area was as high as three forths of the concentration in the control area. This discrepancy can partly be explained by differences in calculated intakes of certain foodstuffs. For example, daily rice intake for an adult in most of the areas mentioned in Table 6:4 is 350 g - 400 g. In the polluted area of Annaka, though, the intake is only 267 g and in the corresponding control area 281 g. The difference in the results of the NNC method depends on differences of cadmium intake via wheat and vegetables. The relation between the concentration of cadmium in rice and in other foodstuffs such as wheat, sweet potatoe, vegetables etc., varied greatly. In the polluted area of Kiyokawa sweet potatoe and wheat had three times higher cadmium concentrations than rice (Japanese Association of Public Health, 1970b), but on the other hand, in the polluted area of Tsushima the rice concentration was 4 times higher than that of wheat and 1.5 times higher than that of sweet potatoe. The geographical features and agricultural methods of the polluted area may explain such great differences.

A way to validate the methods would be to study daily fecal excretion of cadmium as discussed in section 3.2. A comment that also should be made is that the daily intakes calculated for some of the control areas (Table 6:4) are considerably higher than the estimated average for non-polluted areas of Japan, 60 µg/day (Ministry of Health and Welfare, 1969). In the control area of Annaka this is reflected by a very high concentration in rice, 0.3 ppm.

6.3 CADMIUM EXPOSURE AND MEDICAL EFFECTS IN INDIVIDUAL AREAS

6.3.1 Fuchu in Toyama Prefecture

In this area Itai-itai disease was first found. The rice fields were irrigated from the Jintsu river, which had been contaminated by waste products of the Kamioka mine. Ishizaki, 1969, as quoted in CITE, found that proteinuria and glucosuria were more common in the Itai-itai disease endemic area than in a control area (Figure 6:1). Further epidemiological studies in Fuchu have been performed (Fukuyama and Kubota, 1972a, b and c) on the relation between the level of heavy metal pollution and proteinuria respectively glucosuria.

Paddy soil from a number of places within the Itai-itai disease endemic area was analyzed for the content of heavy metals by a Research Committee for the Itai-itai disease in 1968 according to Fukuyama and Kubota, 1972c. They used these values for estimating exposure levels of Cd, Pb and Zn in 12 villages along 4 different irrigation canals (Figure 6:4). The villages here mentioned are in some cases a combination of two to three hamlets, where populations were small. The concentrations of lead in the soil are about 100 times higher than the concentrations of cadmium

(Figure 6:6). On the other hand, Tsuchiya, 1969, reported about rice samples from the Itai-itai disease endemic area average values of lead concentration about 0.2 ppm and cadmium concentration about 0.8 ppm.

Ishizaki, Fukushima and Sakamoto, 1968, analyzed the cadmium concentration in rice from the Fuchu area (Figure 6:5). In rice from paddies irrigated by the Jintsu river values over 2 ppm were recorded and in the most polluted district values under 0.5 ppm were very uncommon.

Fukuyama and Kubota, 1972c, examined women over 40 years of age (79.7% participation of the women living in the villages). As shown in Figure 6:6 proteinuria (Sulfosalicylic acid method) and glucosuria (Testtape) were well correlated to metal concentrations in soil. These analyses were performed on a blind basis (Kubota, personal communication). Itai-itai disease patients had an excretion of lead in urine within normal ranges (Tsuchiya, 1969).

In another study an undefined target population of men and women (Fukuyama and Kubota, 1972b) was examined with the G-25 gel filtration method and disc electrophoresis (blind study according to Kubota, personal communication). This time the villages were divided into three groups with different levels of metal concentration in soil.Tubular patterns in disc electrophoresis (disc +) were more common among the persons with longer exposure time and higher age. Given the same exposure time, such patterns were more common among persons living in high-exposure areas (Fukuyama and Kubota, 1972b). From the data it can also be calculated that the G-25 protein quotient increases with exposure time (living time in the area) (Table 6:5) and with exposure level (pollution level) for those older than 40 years.

Shiroishi, Tanii and Kubota, 1972 studied proteinuria in 161 persons (aged 6-81 years) from a non-polluted area of Toyama Prefecture (O-area) and 105 persons (aged 59-92 years) from a home for the aged in Toyama city. Proteinuria was measured quantitatively with the Kingsbury-Clark method and also disc electrophoresis was performed. The persons from O-area were divided into two groups according to age, over and under 60 years. The distribution of different protein concentration levels in urine is shown in Figure 6:7. A protein concentration over 10 mg/dl occurs in about 5% of both the older and younger persons from O-area but in 20% of the persons from the home for the aged. In Table 6:6 is shown the prevalence of different patterns on disc electrophoresis. Among the young group from O-area 95% have normal patterns as compared with 87-89% in the other groups. About 7% have glomerular patterns both among the old in O-area and those from the home for the aged in Toyama. 2% Itai-itai patterns are found in this latter group, but none in the other two groups. These data indicate that disc electrophoresis patterns of the tubular type (Itai-itai-type) are uncommon among "normal" elderly people in Japan. It was not mentioned whether or not the study was performed on a blind basis.

The average daily cadmium intake was not calculated in the recent epidemiological studies in Fuchu. The effects of cadmium exposure can therefore only be assessed qualitatively - higher exposure gives higher frequency of tubular proteinuria and glucosuria. Since the analyses were carried out blindly the studies by Fukuyama and Kubota mentioned in this section, have an advantage over most other recent Japanese epidemiological studies on the effects of cadmium.

6.3.2 Tsushima in Nagasaki Prefecture

Four villages with a total population of about 1000 are spread along two rivers polluted with cadmium and other heavy metals from mining operations (Figure 6:8). The mines in the area were started in 674 but large-scale production did not start before World War II. The volume of zinc production increased from 1,000 tons in 1948 to 21,000 tons in 1958 and has been relatively stable (22,000-29,000 tons) since then (Terada, pers. comm.).

In CITE an epidemiological investigation from 1969 was presented in detail. Similar investigations have been performed in 1970, 1971 and 1972. Unfortunately, the methods used and the presentation of results are not the same in the different studies. Only parts of the control area used in 1969 have been studied in the later studies and in 1972 no control areas at all were studied according to the report available to us (Nagasaki prefecture, 1972 a).

According to 5 different studies the rice levels of cadmium are at least 6 times higher in the polluted areas (Kashine, Shimobaru, Komoda, Shiine) than in the control area (Are) (Table 6:7). The average daily cadmium intake for adults has been calculated (Japanese Association of Public Health, 1970 b) with the National Nutritional Census method to be 215 µg in the polluted villages and 59 µg in the control village Are (Table 6:4). The Total Diet method on a sample of 8 persons from each of the polluted area and the control area gave 213 µg resp. 104 µg.

The study from 1969 quoted in CITL showed that the prevalence of proteinuria (Testtape) (Takabatake, pers. comm.) was higher in polluted villages than in control villages (Are and Hikage-kamiyama) (Figure 6:2). In 1970 547 persons over 30 years of age from the polluted area (participation 43% of the target population) and 120 persons from a control village (Hikage-kamiyama) (participation 27%) were studied (Nagasaki Prefecture, 1971). The reported prevalence of proteinuria (Trichloracetic acid method + or higher, according to Terada, pers. comm.) was 4.9% and 0.8% respectively. The difference was statistically significant.

In 1971 882 persons in the same age groups were examined in the polluted area (participation = 81%) and 5.3% had proteinuria (Nagasaki Prefecture, 1972 b). In the control area (Hikage-kamiyama) 177 were examined (participation = 43%) and 4.5% had proteinuria (Trichloracetic acid method) (Nagasaki Prefecture, 1972 b). The studies in Tsushima have not been carried out on a blind basis (Terada, pers. comm.).

Age-related proteinuria prevalences have been reported by Terada, pers. comm. The data generally show an increase in proteinuria with age. The control groups studied are very small. No significant differences between control and polluted areas within 10-years age groups can be found. The situation in Tsushima is difficult to evaluate due to inconsistencies in methods used and the low participation.

6.3.3 Ikuno area of Hyogo prefecture

The source of cadmium in this area is a copper and zinc mine, which dates back to 807 and which has had a fairly constant production during the last decades. The first epidemiological study was performed in 1971 on 1676 persons over 30 years of age living in a number of villages along the Ichikawa river (Watanabe, pers. comm.). The aim of the study was to find suspected cases of Itai-itai disease. In 1972 the same area was studied once more regarding proteinuria (Hyogo Prefecture, 1972).

In February 1971 cadmium pollution was detected in this area. Average cadmium concentration in rice produced in 1970 and 1971 was studied by the agricultural bureau of Hyogo Prefecture (according to Ikuno Mining Poll., 1972).x) The average cadmium concentration in 214 samples of rice from 1970 was 0.50 ppm and in 150 samples from 1971 0.39 ppm. The concentrations of cadmium in rice at various places along the Ichikawa river were mapped (Figure 6:9), although it was not mentioned in the report just how the samples were chosen. The figures along the river are average values within different towns along the river as reported by Ikuno Mining Poll., 1972 (see Table 6:8). We have no detailed data from Hyogo Prefecture, but the magnitude is in accord with data from Watanabe (pers. comm.).

In Ikuno no studies on daily cadmium intake have been performed. Based on the rice method (see section 6.2.5) it can be calculated that in Ikuno town (I) the average daily intake would be around 420 µg(Cd in rice = 0.69 ppm) and in Kamisaki (K) town 290 µg (Cd in rice = 0.48 ppm).

The "polluted" area in the 1971 epidemiological study (Watanabe, pers. comm.) was selected according to the standard method (see section 6.2.1). A number of villages, where average cadmium concentration in urine of inhabitants was more than 9 µg/l, were designated as the area requiring health examinations. 1676 of the 1700 persons over 30 years of age living in these villages were examined in 1971 with a modified first screening for Itai-itai disease (cf. section 6.2.2) consisting of interviews, measurement of blood pressure and proteinuria (Trichloracetic acid method). 21.9% of those examined had proteinuria (+ or higher). No data from a control area were reported.

+) This reference was published by the "Ikuno Mining Pollution Prevention Professional Group", which is an anti-pollution citizen group. Efforts are being made to verify the exposure data directly by Hyogo Prefecture and indirectly by Dr. Tsuchiya.

A new epidemiological study on the same target population was performed in 1972 (Watanabe, pers. comm.). This time 3 control areas were also examined. According to Watanabe (pers. comm.) cadmium concentration in rice in the control area A ("Kaibara") was considered to be less than 0.1 ppm. The epidemiological study of 1972 was performed by the Hyogo Prefecture Committee for the study of cadmium poisoning, of which Professor Kitamura is chairman. The Committee's accumulation of data was presented to the study group for differential diagnosis of cadmium poisoning and Itai-itai disease on September 6, 1972 by Dr. Watanabe. The method used for proteinuria analysis in 1972 was the Sulfosalicylic acid method and the results are shown in Table 6:9 (Hyogo Prefecture, 1972). The polluted area (Ikuno) has a strikingly higher prevalence of proteinuria than two of the control areas and the prevalence is also considerably higher than in the third area. The studies were not carried out blind. On the other hand the magnitude of the difference is such that it seems difficult to explain the differences between polluted area and control area by methodological factors.

In order to evaluate the differences in prevalence of proteinuria Watanabe studied a random sample of 376 persons from Ikuno and 250 persons from "Kaibara". In these samples prevalence of proteinuria (Trichloracetic acid method) was 18% resp. 12%. The results of Sulfosalicylic acid method in the whole population were about 3 times as high. As the methods should give fairly similar results (see section 6.2.4) there is reason to believe that the samples were not representative of the whole population as far as proteinuria is concerned. The geometrical average protein concentration in urine (Biuret method) was 2.19 µg/dl (exposed) resp. 2.42 µg/dl (control)(Hyogo Prefecture, 1972). In the sample a lot of normal urines were included, which has a strong influence on the geometrical average. Similar studies were not carried out in the two control areas with lower prevalence of proteinuria.

The high prevalence of proteinuria in Ikuno has been confirmed by Ishizaki et al., 1972. He studied 18 persons over 50 years of age (all farmers, both sexes, who had lived more than 30 years in the area) from Mayumi (Figure 6:9), one of the most polluted villages. 56% had proteinuria (sulfosalicylic acid method) and 39% concurrent proteinuria and glucosuria (testtape).

Watanabe et al., 1972, have shown that the globulin/albumin ratio is commonly elevated among persons with high cadmium exposure and with high protein- and cadmium concentrations in urine. Elevated globulin/albumin ratio reflects tubular proteinuria and is suggested by Watanabe et al., 1972 as an index for screening of cadmium induced proteinuria. Such studies have been carried out by Watanabe in areas with different degree of cadmium pollution.

For 6 villages with different levels of cadmium exposure data on urinary cadmium and globulin/albumin ratio have been compiled (Watanabe, unpublished) (Table 6:10). The age distribution of the persons sampled was not reported. There was a significant difference in globulin/albumin ratio between persons living in villages with 0.33-0.35 ppm average cadmium concentration in rice and those with 0.61-1.10 ppm. Urinary cadmium concentrations increased distinctly with increasing cadmium in rice.

6.3.4 Kakehashi area in Ishikawa prefecture

The source of cadmium in this area is nowadays the Hokuriku mine and was earlier also the Ogoya mine (Figure 6:10). An epidemiological study has been performed by Ishizaki, 1972. A total of 209 persons over 50 years of age from four villages were studied for proteinuria, glucosuria and cadmium in urine. No non-polluted area was included. Results of the study together with cadmium concentrations in rice are given in Table 6:11.

The village Kanahira closest to the mine, is the most polluted with an average cadmium concentration in rice of 0.80 ppm

(Table 6:11). In the other villages the concentrations were between 0.23 and 0.34 ppm. Average urinary cadmium concentration was 23.3 µg/l in the most polluted village. Urinary cadmium concentrations were also high in the other villages. Prevalence of proteinuria (3% Sulfosalicylic acid method) was 39% in the most polluted village and 22-30 % in the other villages. Ishizaki, 1972, considers positive reaction with this method to occur at 5 mg protein/dl urine. It is not clear how this evaluation was made. Concurrent proteinuria and glucosuria was twice as high in the most polluted village as in the other villages.

Urine protein electrophoresis on cellulose acetate membrane was performed in several cases. The results of these examinations are shown in Figure 6:11. Tubular proteinuria was a common finding and clearly pathologic electrophoresis patterns were almost always of the tubular type. Ishizaki, 1972, concluded that the findings in Kanahira village are similar to those in the Itai-itai district, even though he did not find signs of actual Itai-itai disease. He considered that signs of cadmium effects had been shown in the other three villages as well.

6.3.5 Bandai area in Fukushima Prefecture

Bandai town in Fukushima Prefecture has been polluted by cadmium via air and sewage water outlets from a zinc refinery. An epidemiological survey according to the old standard method was performed in 1970. On those who did not undertake the screening in 1970, follow-up studies were performed in 1971 and 1972, but since very small groups were included the results are not discussed here.

The refining of zinc was started in this town in 1916 and lead production in 1940. At present also cadmium and other substances are produced. In 1962 zinc production was 2,200 tons per month (Nisso Smelting Co., 1972) in 1965 2,500 tons per month and in 1970 4,000 tons per month (Fukushima Prefecture, 1972).

Cadmium concentration in unpolished rice from 1970 and 1971 within the polluted area varies between lower than 0.2 ppm to higher than 1.0 ppm (Fukushima Prefecture, 1971 a).

The control area for the proteinuria study has been the village Kitaaizu, which is 10 km southwest of Bandai. If no special contamination exists it can be assumed that cadmium in rice is not higher than 0.1 ppm in this village.

The target population in the epidemiological study was 1827 men and women over 30 years of age in the polluted area and 272 women in the control area (Kitaaizu). The average prevalence of proteinuria among the 1324 participants from the polluted area in 1970 was 12.3% and among the 215 in the control area 4.7% (Fukushima Prefecture, 1971 b). Nothing is mentioned as to whether or not the studies were carried out on a blind basis. Data on age distribution are available for those who underwent the second screening. The criteria for selecting persons for the second screening was proteinuria ± in the polluted area and proteinuria + in the control area. Further in the polluted area also persons with glucosuria were included. This makes it impossible to compare age specific prevalences for epidemiological analysis.

5.3.6 Annaka area of Gumma Prefecture

In Annaka a large zinc refinery has polluted surrounding farmland with cadmium primarily through air. Operations started in 1937 and cadmium production dates from 1948. The monthly production of zinc has increased rapidly during the last decade (Figure 6:12).

An epidemiological study was performed in 1969-1970 (Kakinuma et al., 1971) according to the old standard method. Average cadmium concentration in rice in 1968 was 0.38 ppm in the polluted area and 0.30 ppm in the "control" area (Table 6:4). In 1971, paddy soil and unpolished rice were studied in the polluted area and surrounding areas (Gumma Prefecture, 1972).

An average of 0.48 ppm cadmium in 166 samples of unpolished rice in the polluted area was found. The "control" area was not studied, but in a village 1 km from the "control" area and considered to be non-polluted, the average cadmium concentration out of 3 samples was 0.48 ppm. It can therefore be assumed that the similarity between the polluted and the "control" area in cadmium concentration of rice in 1968 was not accidental. It may even be argued that the "control" area in Annaka seems to have a similar cadmium exposure as the polluted area. Neverthele it was calculated with the National Nutritional Census method (Japanese Association of Public Health, 1970) that daily cadmium intake was 211 µg in the polluted area and 113 µg in the "control" area (see section 6.2.5). This was due to an assumed low cadmium concentration in other foodstuffs from the "control" area.

The "control" area used in the studies on daily intake is the same as the one used in the epidemiological study, where a total of 2397 persons (men and women over 30 years) in the polluted area (84% participation) and 895 persons in the "control" area (74% participation) were studied. Age-related prevalences of proteinuria have been published by Kakinuma et al., 1971, and are shown in Table 6:12. The studies in the polluted area and in the "control" area were not performed at the same time and not on a blind basis (Nomiyama, pers. comm.). According to Kakinuma et al., 1971, proteinuria was defined as a rating of + or higher in either the Trichloracetic acid method or the Sulfosalicylic acid method.

The data in Table 6:12 do not show differences in the age-related prevalence of proteinuria between the "control" and the polluted area. It should be pointed out, however, that the difference in cadmium exposure judged by the concentration of cadmium in rice seems to be trivial. There is an increase in prevalence of proteinuria with age.

Another factor of importance is that the production of the factory has increased 6-fold during the last decade (Figure 6:12) and has been high only the last years. Thus the high values in rice do not necessarily implicate a high cadmium exposure in the past. In the Annaka area there must have been an exposure also via ambient air. The magnitude of such exposure has not been possible to evaluate.

6.3.7 Omuta area in Fukuoka Prefecture

The cadmium source in this area is a refinery causing mainly airborne pollution. Epidemiological data have been collected in 1970 (Ministry of Health and Welfare, 1971 c) and in 1972 (Matsushiro, pers. comm.).

The refinery started production in 1913 and two plants were added in 1915 and 1954. Data from the Ministry of Health and Welfare, 1971c, indicate that only a slow increase in production has occurred the last decades. Air concentrations of cadmium in 1969 were the highest measured in Japan (Yamamoto, 1972). At one place a maximum 24-hour value of 3 $\mu g/m^3$ was found. According to Yamamoto, 1972, the average cadmium concentration in 91 samples of rice was as high as 0.72 ppm. Intake from all food except rice was calculated to be 75 µg to 115 µg among farmers, depending on area. Total daily intake has been calculated to vary between 60 µg and 280 µg (Ministry of Health and Welfare, 1971c). Using the rice-method (described in section 6.2.5) the daily intake can be calculated to be about 500 µg.

Prevalence of proteinuria was studied in the polluted area in 1970 and 1972 (Ministry of Health and Welfare, 1971c, and Matsushiro, pers. comm.) and in 1970 in a control area, Fukuoka city. The exposure to cadmium in the control area is not known. Information on methods used for protein determination has not been available to us for the 1970 study. In 1972 Trichloracetic acid or Sulfosalicylic acid method was used (Matsushiro, pers. comm.). The selection of study populations is not known to us nor are the participation rates. With these reservations the prevalences of proteinuria in polluted as well as control area are given in Table 6:13. In the polluted area the prevalence increases with age both in 1970 and 1972. The control area studied in 1970 showed higher prevalences than the polluted area (1970).

In the report from the Ministry of Health and Welfare, 1971 c, many values on cadmium in urine are given, but not information on methods. The data are given without reference to age. It can be mentioned that the group with the highest cadmium excretion consisted of farmers with proteinuria. These 130 persons had an average cadmium excretion of 10.3 µg/l (range 0-77.7 µg/l). Unfortunately, no information on cadmium excretion in farmers without proteinuria was given. Those with proteinuria in the control group (49 persons) had an average cadmium excretion of 1.8 µg/l (0-5.1 µg/l). In the control area there were reported data on cadmium excretion among those without proteinuria. These values are not given as concentrations, but as total amounts excreted per day. A mean of 2.8 µg/day (range 0-7.5 µg/day was reported among 38 persons.

6.3.8 Uguisuzawa area in Miyagi Prefecture

Epidemiological studies have been performed in connection with pollution of Uguisuzawa town. In 1969 638 persons of the "most polluted" area, 220 persons of a neighboring area and 238 persons in a control area underwent a first screening according to the old standard method (see section 6.2.2) (Miyagi Prefecture, 1970a). The prevalence of proteinuria (Trichloracetic acid method) in women over 30 years of age was as follows: 50% in the "most polluted" area, 60.4% in the neigboring area and 39.6% in the "control" area. In 1970 a new study using "testtape" (which is less sensitive for tubular proteinuria) was performed (Ichinowatari, pers. comm.). This time only 117 persons were studied in the "most polluted" area and the proteinuria prevalence was 3% (Hasegawa, 1972). A new control area was used, from which 437 persons were studied and the result was 4% proteinuria (Hasegawa, 1972).

In Uguisuzawa there are a mine and a refinery, which have polluted both air and water. The water of a small river passes the "most polluted" area close to the mine at a rapid pace and then converges with another river and flows more slowly through the neighboring area (Ichinowatari, pers. comm.), which lies about 10 km downstream from the mine. The river continues another 10 km and passes through the control area used in 1970 and then after another 10 km passes through the control area used in 1969. This distance would not at all exclude cadmium contamination. Note that the distance from the Kamioka mine to the polluted Fuchu area in Toyama Prefecture is about 30 km.

A nutritional study from 1969 (Miyagi Prefecture, 1970b) has calculated the daily intake in the polluted area (including the neighboring area, Ichinowatari, pers. comm.) to 347 µg cadmium and in a control area to 80 µg (Table 6:4). This control area, however, is not the same as the ones used in the epidemiological studies and cannot be contaminated by the river flowing from the mine.

6.4 DISCUSSION

A large amount of epidemiological data has been collected during recent years in various parts of Japan, where cadmium pollution of the environment has been suspected. A standard screening method has been established by the Ministry of Health, but the major aim of this method is to find cases of Itai-itai disease. In other words, the screenings are more individual-oriented than population-oriented.

The way of delineating "polluted" and "control" areas has not been standardized. In some control areas, the exposure has not been estimated, and in at least one area it can be assumed that also the "control" area was polluted.

Methods for proteinuria analysis vary among areas, and a choice between Trichloracetic acid and Sulfosalicylic acid method is explicitly allowed by the standard screening method itself. Though the standard screening method sets criteria for evaluating proteinuria, very few of the published studies give detailed data on qualitative ratings (-, ±, +, ++, +++) of proteinuria. The prevalences of proteinuria are as a rule only based on whether or not proteinuria was present. Furthermore, in some reports proteinuria has been based on a rating ± or higher and in other studies + or higher.

The results of the proteinuria studies vary greatly among different areas. From what was just mentioned it is clear that differences in analytical methodology may play a large role. Another reason may be the effect of expectation. If the studies are not blind and the analyst knows that the sample is from a polluted area or a control area, he/she might unconsciously use different criteria for the two areas. This effect may work in either direction, which means that an existing difference may be hidden or a non-existing difference may be created. When using the Trichloracetic acid or Sulfosalicylic acid methods the critical part of the evaluation is the discrimination between a rating of ± or higher and + or higher. Judging from the methodology studies published (see section 6.2.4) the effect on prevalence of proteinuria of errors in this discrimination in certain studies may be very high.

The only attempt to evaluate information from different areas has been made by Hasegawa, 1972. He concluded that no effect of cadmium could be ascertained with the epidemiological analysis he used. According to him, this lack of effect might depend on level of exposure, i.e. not high enough, or a lack of a sensitive method. Unfortunately, he only used the classification "polluted" versus "control" as a means of estimating the population's exposure. As shown in section 6.3 the population of the control area is not necessarily unexposed. Furthermore, Hasegawa did not study age-related effect data.

In most polluted areas for which age-related proteinuria prevalences have been possible to calculate (Fuchu, Tsushima, Annaka) there is a substantial increase in prevalence with age. The new data from Fuchu certainly confirm the earlier conclusion in CITE that in that area the causal role of cadmium for increased prevalence of proteinuria with increase in age is obvious. It is not possible in the other areas with the data available to assess whether or not the increase with age is also causally related to the longer exposure to cadmium. The main reason for this uncertainty is that virtually no data exist from true control areas on prevalence of proteinuria within different age groups as analyzed by the same methods applied in the polluted areas.

When it comes to an evaluation of those areas where no age specific data are available the following can be said. In for example Ikuno, Uguisuzawa and Kakehashi, where extensive studies have been carried out, prevalence of proteinuria was found to be about 40-60%. This certainly means that the occurrence of proteinuria was higher than usual in Japan. In Ikuno the polluted area showed a prevalence of about 60% (in people above 30 years) while in two control areas corresponding figures were about 4 and 9%. It is true that in another control area an average value of 33% was reported. In Kakehashi no control area was examined while in Uguisuzawa the control showed about the same prevalence of proteinuria as the exposed area. This control area, however, was located along the same river, which had polluted the exposed area. Other studies, like the one in Annaka, have clearly shown that a control area may be as contaminated as a polluted area.

In Fuchu and Bandai within the same studies it was possible to find either a clear dose-response relationship or a difference compared with a control area. The data from Omuta are difficult to interpret. In one study the data indicate a lower prevalence of proteinuria in a cadmium-polluted area than in a control area.

A question of importance of course is whether the daily intakes (between 200 µg and 500 µg) could be considered sufficient to give chronic cadmium poisoning. If it is assumed that an exposure of 50-75 µg/day during 50 years gives about 50 ppm in kidney cortex a corresponding exposure to 200-300 µg/day would give about 200 ppm as an average. Low calcium intakes or vitamin D intakes would enhance Cd-absorption and lower daily intakes than 200 µg might then give effects.

Another approach in evaluating effects is to discuss cadmium excretion in the urine. It can be seen from data by Tsuchiya, Seki and Sugita, 1972 a (section 4.3.4.1) that a mean urinary excretion of about 2 µg Cd/liter corresponds to a mean cadmium level in kidney cortex of about 100 ppm after long term low level exposure in a so called non-polluted area. As was discussed in section 4.3.4.1 at this type of exposure there is reason to believe that urinary excretion is related to body burden. It has not been shown that a threshold exists, at which the urinary excretion of cadmium suddenly would increase without concurrent tubular dysfunction. As was discussed in section 5.1.3 the critical cadmium level in kidney cortex at which proteinuria might occur was estimated to be 200 ppm. The corresponding urinary excretion would, if it is assumed that kidney cortex concentration is proportional to body burden, be 4 µg Cd/liter (200/100 x 2). In for example the study in Ikuno the criteria for selecting the polluted areas was a minimum average cadmium excretion in urine of 9 µg/liter. In two villages in Kakehashi, mainly among persons with proteinuria, the cadmium concentration in urine was on an average about 22 µg/liter. Even if the urinary excretion is not directly related to kidney cortex concentration in the same way as to body burden, all human as well as animal data strongly support tubular dysfunction associated with long-term cadmium exposure as the reason for such a high excretion.

In our report Cadmium in the Environment it was stated that there was reason to believe that cadmium intoxication had occurred in at least one part of Japan other than the Toyama Prefecture and that the cadmium problem in Japan might well be far reaching. Though the new data presented in this report are often difficult to interpret due to sometimes severe methodological inconcistencies there can be no doubt that the conclusion reached in our earlier report has been strengthened considerably.

TABLE 6:1 Result of comparison of proteinuria methods in two areas of Toyama. (From Fukuyama, Shiroishi and Kubota, 1971).

Total number examined		Polluted area		Control area	
		165 (100.0)		225 (100.0)	
Sulfosalicylic	+	21 (12.7)	40 (24.2)	3 (1.3)	7 (3.1)
acid method	±	19 (11.5)		4 (1.8)	
	-	125 (75.8)		218 (96.9)	
Trichloracetic	+	13 (7.9)	37 (22.4)	5 (2.2)	25 (11.1)
acid method	±	24 (14.5)		20 (8.9)	
	-	128 (77.6)		200 (88.9)	
G-25 gel fil-	+	63 (38.2)	110 (66.7)	3 (1.3)	6 (2.7)
tration method	±	47 (28.5)		3 (1.3)	
	-	55 (33.3)		219 (97.3)	

Numbers indicate how many people were studied in each group; numbers within brackets are vertical relations (%).

TABLE 6:2 Comparison between disc electrophoresis and other proteinuria methods. (Calculated from a table by Fukuyama, Shiroishi and Kubota, 1971).

	Disc (+)		Disc (±)		Disc (-)		Total	
	N	%	N	%	N	%	N	%
Total	50		42		73		165	
Sulfosalicylic acid method > ± or higher	33	82.5	5	12.5	2	5.0	40	100
Trichloracetic acid method > ± or higher	25	67.6	8	21.6	4	10.8	37	100
SA or TCA > ±	36	67.9	11	20.8	6	11.3	53	100
G-25 gel filtration method +	47	74.6	11	17.5	5	7.9	63	100

Numbers within brackets are horizontal relations (%). N = Number of persons examined.

TABLE 6:3 Result of comparison of proteinuria methods in Annaka. (From Kakinuma et al., 1971).

		35% Trichloracetic acid method (TCA)			
	Rating	+	±	-	Total
3% Sulfosalicylic	+	257	39	17	313
acid method	±	31	233	48	312
(SA)	-		2	1809	1811
	Total	288	274	1874	2436

Figures indicate the number of persons with different combinations of ratings according to the trichloracetic acid method and the sulfosalicylic acid method.

TABLE 6:4 Average daily cadmium intake for adults
(From Japanese Association of Public Health, 1970b).

Area		National nutrition census µg Cd	N	Rice conc. of Cd ppm	Total diet coll. µg Cd	N	Range (total diet coll). µg Cd
Uguisuzawa	polluted	244.7	513	0.30	180	6	119-277
(Miyagi Pref.)	control[x]	84.5	158	0.11	108	6	78-141
Annaka	polluted	211.0	676	0.38	281	6	148-485
(Gumma Pref.)	"control"	113.4	138	0.30	99	6	71-132
Tsushima	polluted	215.2	567	0.37	213	8	49-533
(Nagasaki Pref.)	control	59.1	284	0.08	104	8	58-192
Kiyokawa	polluted	221.6	666	0.18	391	6	117-1209
(Oita Pref.)	control	79.2	224	0.03	-	6	-

[x] not the control area used in the epidemiological studies.

N = number of persons examined.

TABLE 6:5 Average G-25 protein quotient in relation to exposure level and exposure time.
(Calculated from Figure 2 in Fukuyama and Kubota, 1972 b

Exposure time (years)		0-29	20-39	40-59	60+
Exposure level	High	6.4	6.9	15.3	24.2
	Medium	6.7	8.0	14.3	21.4
	Low	7.0	7.1	10.0	17.2

TABLE 6:6 Number of persons in O-area and a home for the aged with different patterns in disc electrophoresis.
(From Shiroishi, Tanii and Kubota, 1972)

Disc electrophoresis pattern

Group studied		N	K	I	O
O-area	young	104 (95.4)	2 (1.8)	-	3 (2.7)
O-area	old	46 (88.5)	4 (7.6)	- -	2 (3.8)
Home for the aged		91 (86.6)	7 (6.6)	2 (1.9)	5 (4.7)

N = normal pattern
K = glomerular pattern
I = Itai-itai pattern
O = other pattern

Figures within parenthesis are percent of the total in group.

TABLE 6:7 Concentration of cadmium in unpolished rice from different villages of Tsuchima.

Village	Range of reported cadmium concentrations from 1969 - 1970[o] (ppm)	Averages Oct. 1970[&&]
Polluted		( ) = number of samples
Kashine	0.38 - 0.89	0.60 (58)
Shimobaru	0.30 - 0.50	0.42 (39)
Komoda	0.36 - 1.32	0.50 (34)
Shiine	0.44 - 0.60	0.58 (52)
Control		
Are	0.50[&]	

& only studied in 1969 (Nagasaki prefecture, 1971 a)

o results from five different studies (Nagasaki prefecture, 1971 a)

&& average of results from a study by the Ministry of Agriculture and Prefectural Agriculture Center, October 1970 and a study by the Ministry of Health and Prefectural Pollution Bureau, October 1970.

TABLE 6:8 Cadmium concentration in rice from the Ikuno area, 1971. (Analyzed by the Agriculture Bureau of Hyogo Prefecture according to Ikuno Mining Poll., 1972)

Area	Number of rice samples	Cadmium concentration (ppm) Maximum	Minimum	Average
Ikuno town (I)	23	1.43	0.15	0.69
Ookochi town (O)	57	1.15	0.01	0.35
Kamisaki town (K)	30	0.98	0.15	0.48
Ichikawa town (IC)	20	0.49	0.01	0.19
Fukusaki town (F)	5	0.37	0.03	0.15
Kooji town (KO)	10	0.72	0.06	0.27

TABLE 6:9 Pravalence of proteinuria in areas of Hyogo Prefecture. Both sexes, all over 30 years (From Hyogo Prefecture, 1972)

			Result of proteinuria analysis. Sulfosalicylic acid method (% of persons examined)		
			Rating		
Area	Target population	Persons examined	+	±	-
Ikuno	1699	1560	58	5	37
A "Kaibara"	1935	1574	33	8	59
B "Chichigawa"	2514	2002	4	5	91
C "Hamasaka"	771	638	9	2	89

TABLE 6:10 Relationship between cadmium concentration in rice, urinary cadmium concentration and globulin/albumin ratio (Watanabe, unpublished data)

Village	Cadmium in rice (ppm)	Number of urine samples	Urinary Cd µg/l	Glob/Alb-ratio
1	0.33	35	7.5±5.2	0.75±0.61
2	0.35	34	8.0±5.1	0.81±0.58
3	0.61	49	9.4±5.3	1.80±0.91
4	0.88	50	12.9±6.1	1.79±0.84
5	0.91	27	13.3±9.0	1.41±0.95
6	1.10	55	13.8±10.7	1.97±0.82

TABLE 6:11 Result of an epidemiological study in Kakehashi river basin (From Ishizaki, 1972).

	Village Kanahira	Gokoji	Kaneno	Shorenji
Cadmium concentrations in rice (ppm) average ± S.D.	0.80±0.30	0.23±0.14	0.32±0.07	0.34±0.28
Persons examined and participation (in brackets)	77(87%)	54(94.7%)	60(87%)	18(100%)
Prevalence of proteinuria (% of persons examined, sulfosalicylic acid method)	39.0	22.0	30.0	28.0
Prevalence of glucosuria (% of persons examined, orthotoluidine method)	33.0	17.0	18.0	11.0
Concurrent proteinuria and glucosuria (% of persons examined)	22.0	13.0	10.0	9.0
Number of persons examined for cadmium in urine	33^{x}	13^{x}	17^{x}	18
Cadmium concentrations in urine (µg/l) average ± S.D.	23.3±11.6	21.2±10.5	11.5±4.8	10.1±6.5

x mainly cases with proteinuria

TABLE 6:12 Prevalence of proteinuria in Annaka area. (Calculated from Kakinuma et al., 1971)

Age group	Females, polluted N	Females, polluted %	Females, "control" N	Females, "control" %
30-39	337	9.8	85	5.9
40-49	351	8.3	156	6.4
50-59	292	11.0	125	10.4
60-69	193	17.6	87	20.7
70 +	128	22.6	54	11.1
Total	1301	12.1	507	10.3

Age group	Males, polluted N	Males, polluted %	Males, "control" N	Males, "control" %
30-39	307	4.2	51	3.9
40-49	273	6.2	115	5.2
50-59	226	6.2	100	13.0
60-69	201	14.9	84	10.7
70 +	89	14.6	38	15.8
Total	1096	7.9	388	9.3

N = number of persons studied

% = prevalence of proteinuria (either Trichloracetic acid + or higher or Sulfosalicylic acid + or higher)

TABLE 6:13 Proteinuria among inhabitants of the polluted area in Gumma and of the control area in Fukuoka. (From Ministry of Health and Walfare, 1971 d, and Matsushiro, pers. comm.)

Female farmers

	Polluted area				Control area	
	1970		1972		1970	
Age	N	%	N	%	N	%
-39	20	0	84	11.9	99	6.1
40-49	419	2.6	102	14.7	126	5.7
50-59	325	2.5	69	11.6	113	6.0
60-69	361	5.8	68	16.2	87	10.3
70 +	310	13.9	94	34.0		
Total	1435	5.8	417	18.2	425	6.8

Male farmers

	Polluted area				Control area	
	1970		1972		1970	
Age	N	%	N	%	N	%
-39	10	0	48	4.2		
40-49	318	1.3	84	7.1		
50-59	262	1.9	76	13.2		
60-69	285	3.2	49	16.3	87	13.8
70 +	230	16.5	57	28.0		
Total	1105	5.1	314	13.4	87	13.8

N = number of persons examined

% = prevalence of proteinuria (1970 method not reported, in 1972 Trichlor acetic acid methods were used, Matsushiro, pers. comm.)

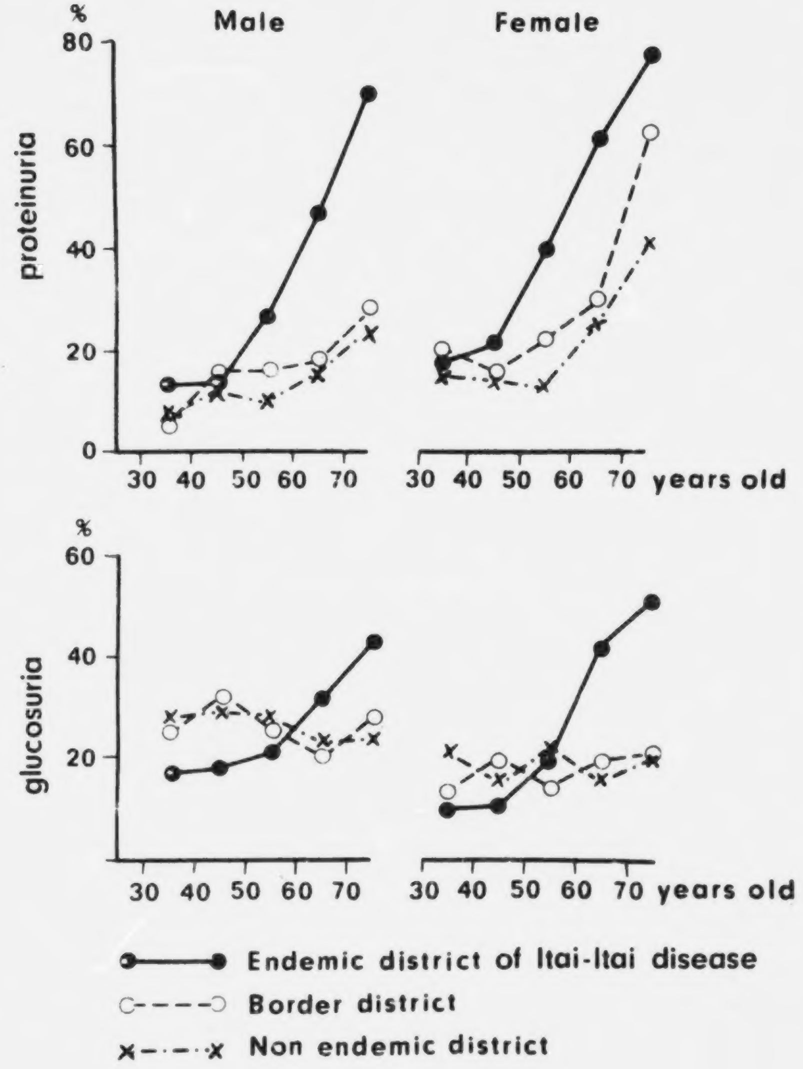


FIGURE 6:1 Prevalence of proteinuria and glucosuria among men and women in different age groups at the 1967 epidemiological study of itai-itai disease in Toyama. (From Ishizaki, 1969).

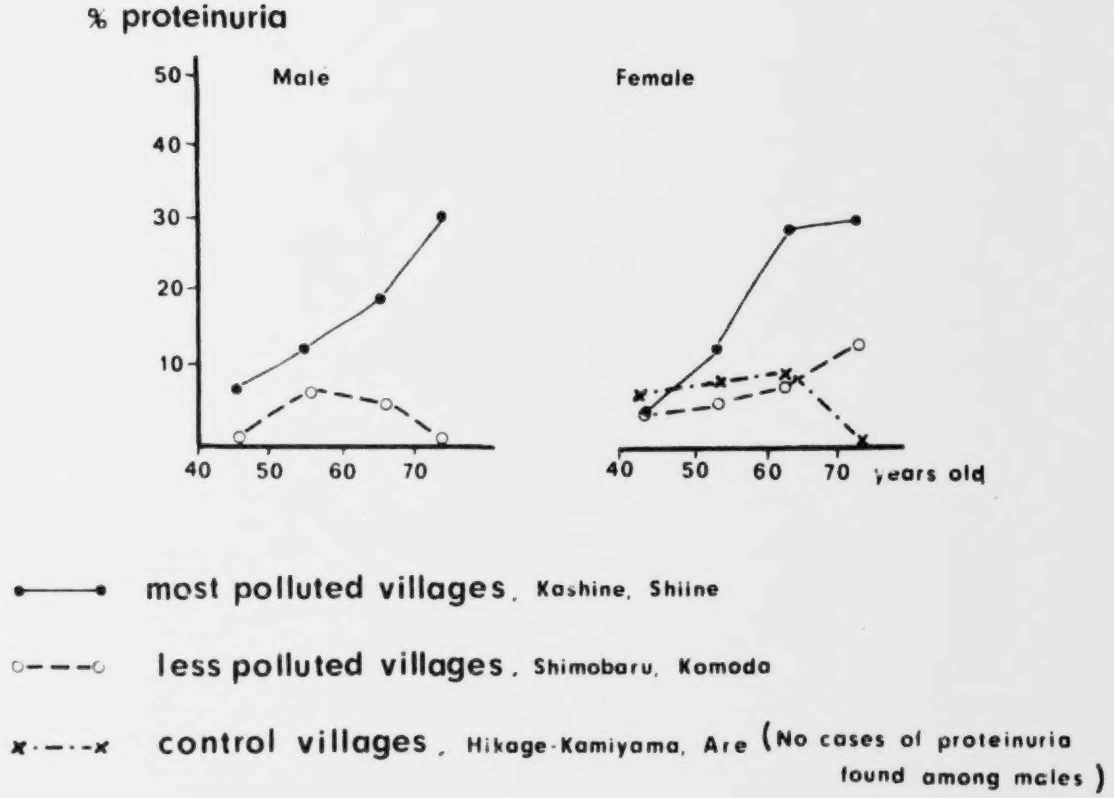


FIGURE 6:7 Prevalence of proteinuria among men and women in different age groups in villages of Tsushima. (From Nagasaki Prefecture, 1970).

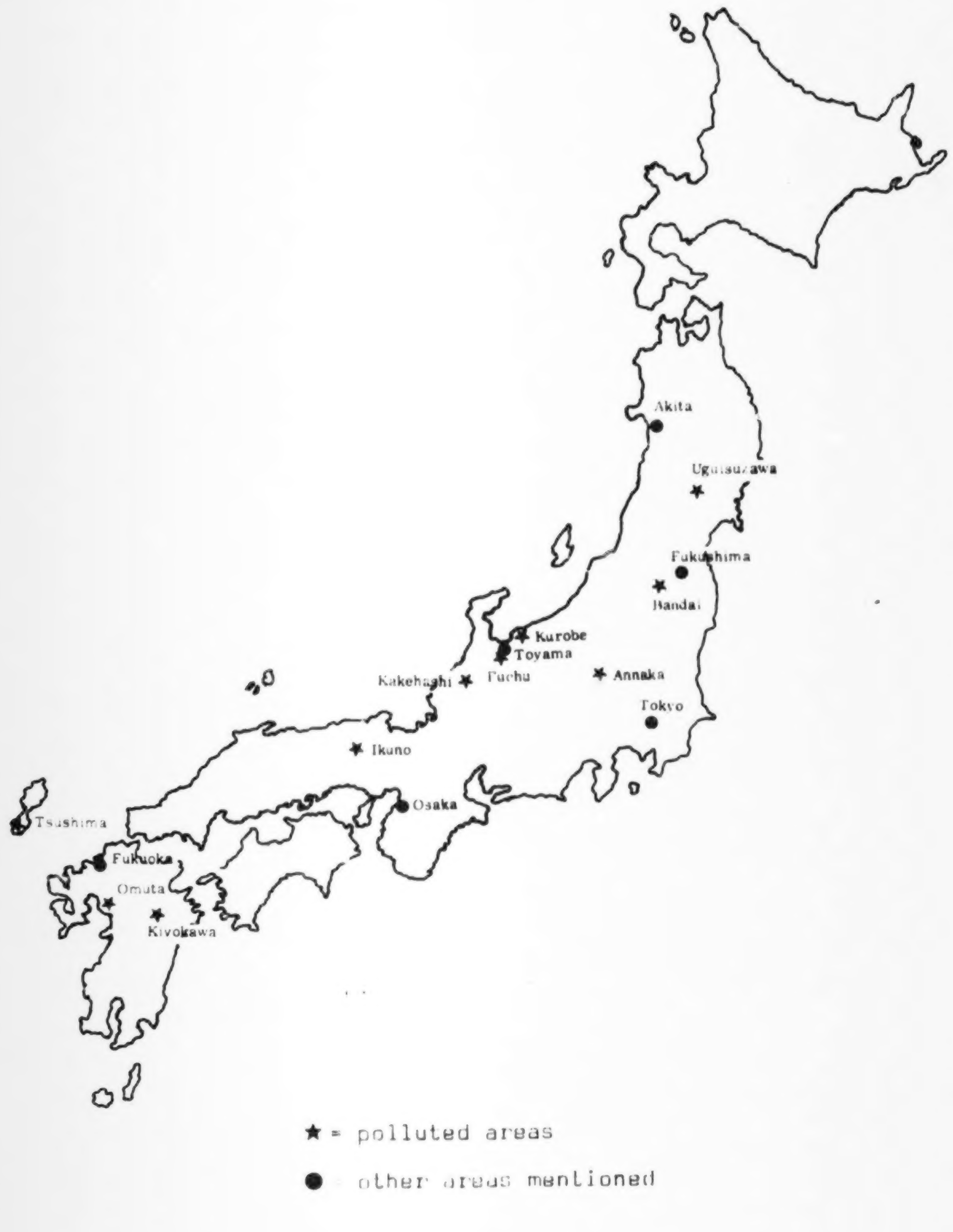
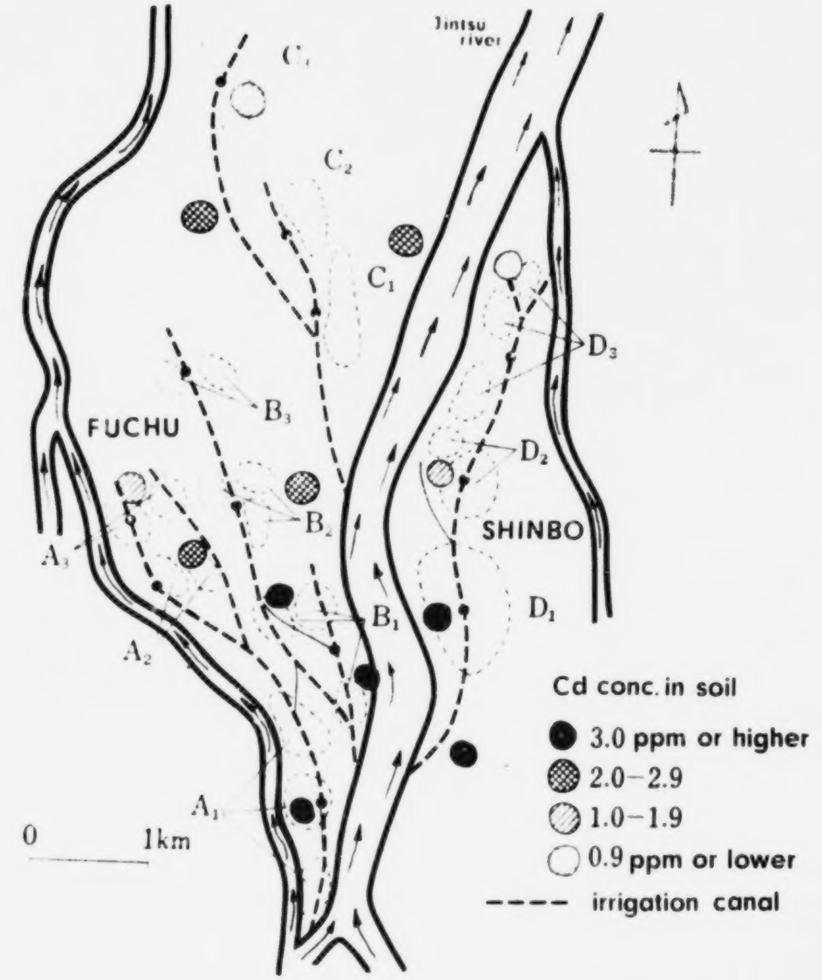

Akita
Uguisuzawa
Fukushima
Bandai
Kurobe
Toyama
Kakehashi
Fuchu
Annaka
Tokyo
Ikuno
Osaka
Tsushima
Fukuoka
Omuta
Kiyokawa
★ = polluted areas
● = other areas mentioned

FIGURE [illegible] Places in Japan mentioned in this report.


Jintsu river
C₃
C₂
C₁
B₃
B₂
B₁
A₃
A₂
A₁
D₃
D₂
D₁
FUCHU
SHINBO
0 1km
Cd conc. in soil
● 3.0 ppm or higher
▩ 2.0−2.9
▨ 1.0−1.9
○ 0.9 ppm or lower
---- irrigation canal

Thin lines show boarders of hamlets included in the study. Cadmium concentration in paddy soil is an average of samples from top soil at water inlet, center and water outlet of paddy.

FIGURE 6:4 Distribution of areas studied, irrigation canals and cadmium in paddy soil. (From Fukuyama and Kubota, 1972b).

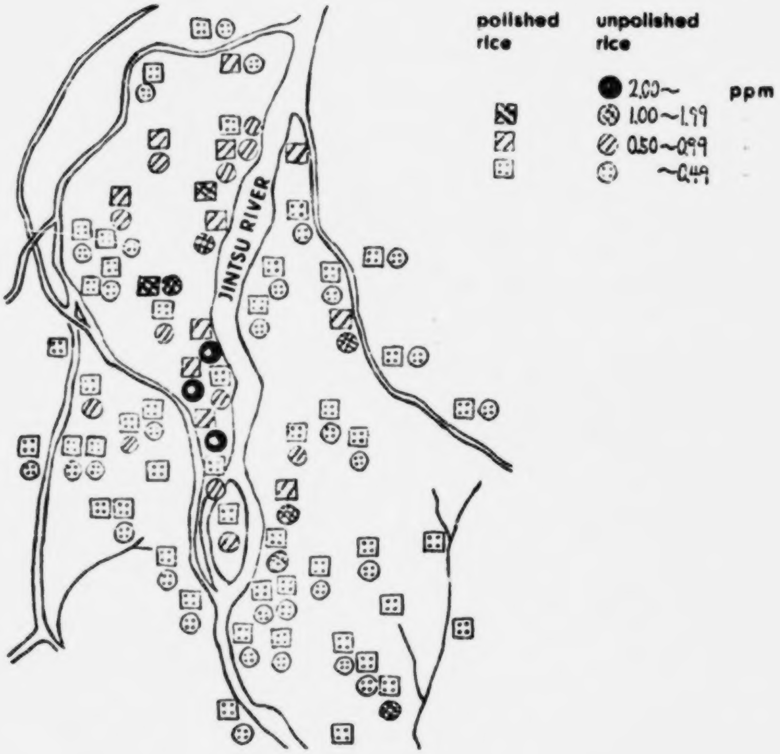

polished rice
unpolished rice
2.00~ ppm
1.00~1.99
0.50~0.99
~0.49
JINTSU RIVER

FIGURE 6:5 Concentration of cadmium in rice in the Fuchu area, 1967. (From Ishizaki, Fukushima and Sakamoto, 1968).

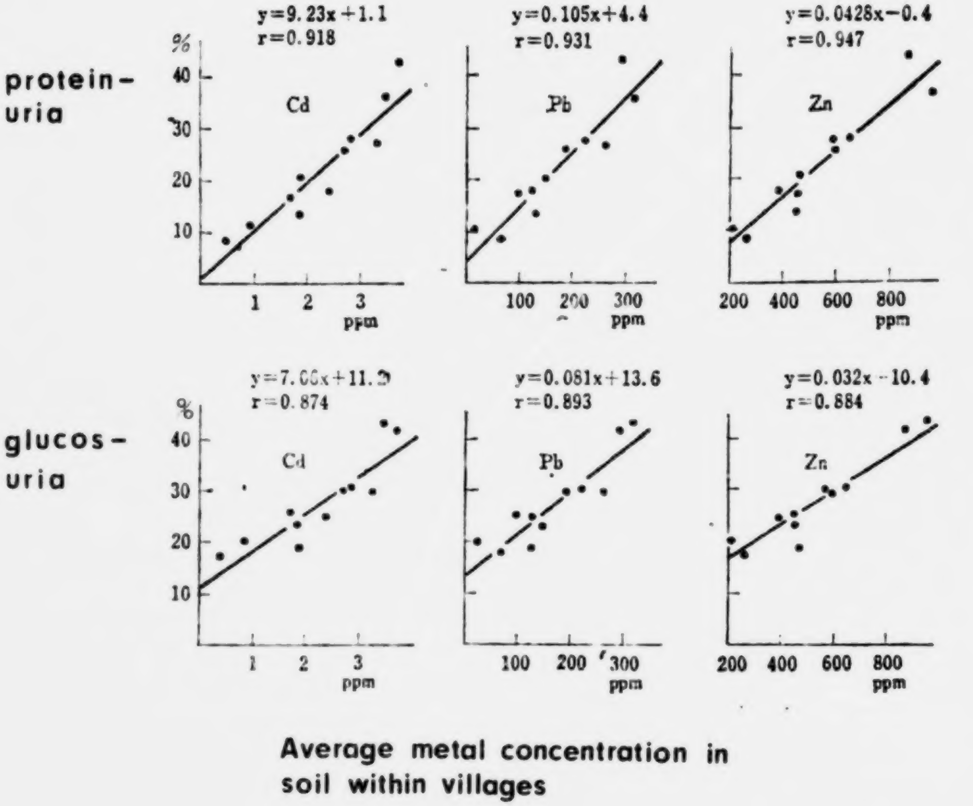
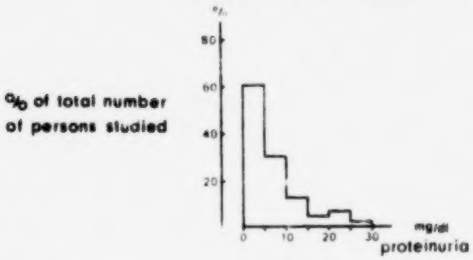
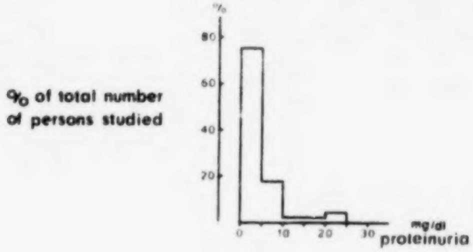


FIGURE 6:6 Village averages of proteinuria and glucosuria in relation to concentration of heavy metals in paddy soil. Regression equations and correlation coefficients. (From Fukuyama and Kubota, 1972c).

Home for the aged in Toyama City



Old group in 0-area



Young group in 0-area

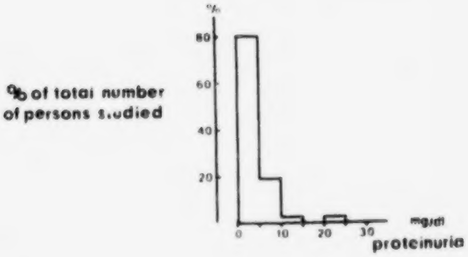


FIGURE 6:7 Distribution of urinary protein concentration levels among people in O-area and a home for the aged in Toyama city. (From Shiroishi, Tanii, and Kubota, 1972).

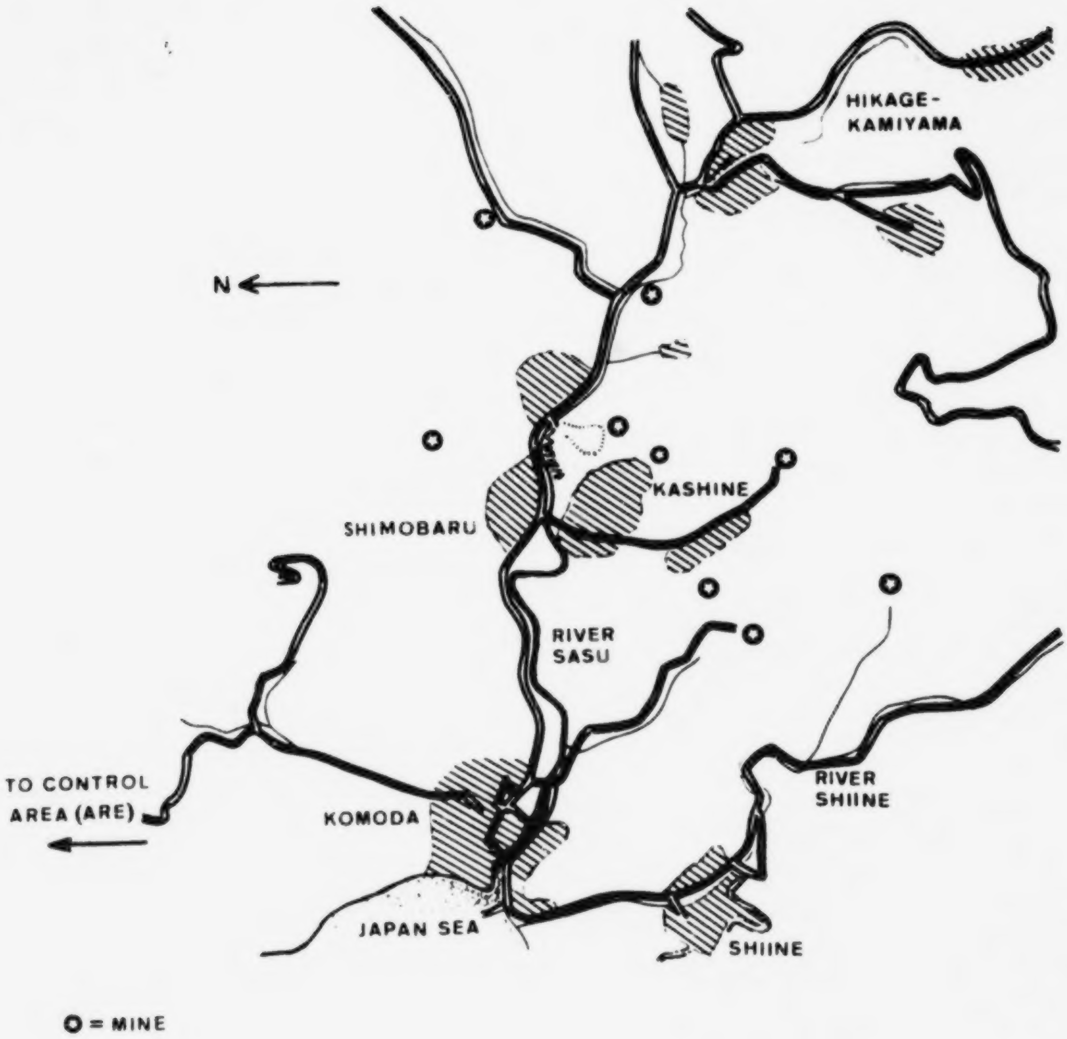
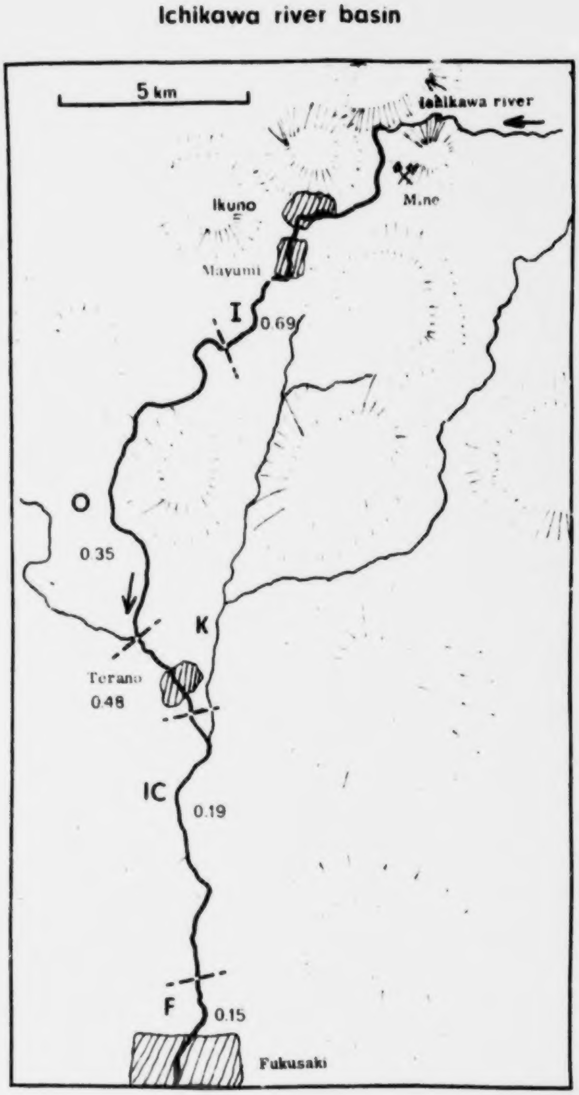

HIKAGE-
KAMIYAMA
N
KASHINE
SHIMOBARU
RIVER
SASU
TO CONTROL
AREA (ARE)
KOMODA
RIVER
SHIINE
JAPAN SEA
SHIINE
O = MINE

FIGURE 6:8 Area under investigation, Tsushima Island. (From Nagasaki Prefecture, 1970).


Ichikawa river basin
5 km
Ichikawa river
Ikuno
Mine
Mayumi
I
0.69
O
0.35
K
Terano
0.48
IC
0.19
F
0.15
Fukusaki

-------- = town borders (I, O, K, IC, F).

The numbers are average cadmium concentrations in rice within towns.

FIGURE 6:9 Map of Ichikawa river and polluted areas in Ikuno area. (Redrawn from a map in Ikuno Mining Poll., 1972).

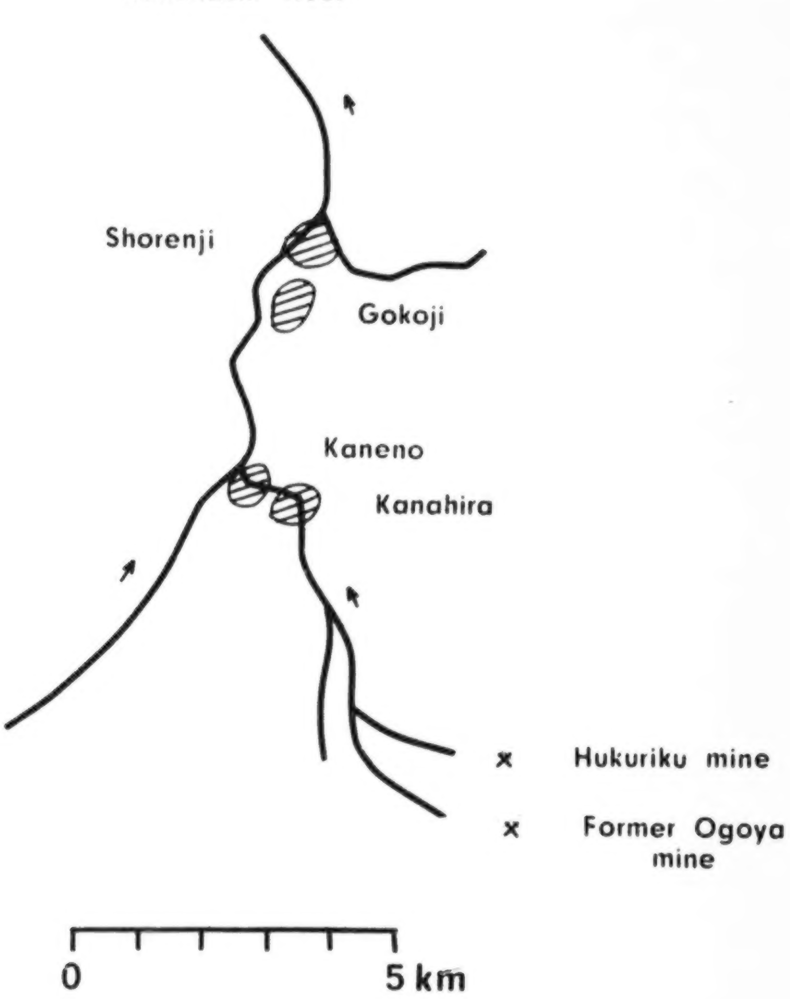

Kakehashi river
Shorenji
Gokoji
Kaneno
Kanahira
× Hukuriku mine
× Former Ogoya mine
0
5 km

FIGURE 6:10 Location of the villages investigated in Kakehashi river basin. (From Ishizaki, 1972).

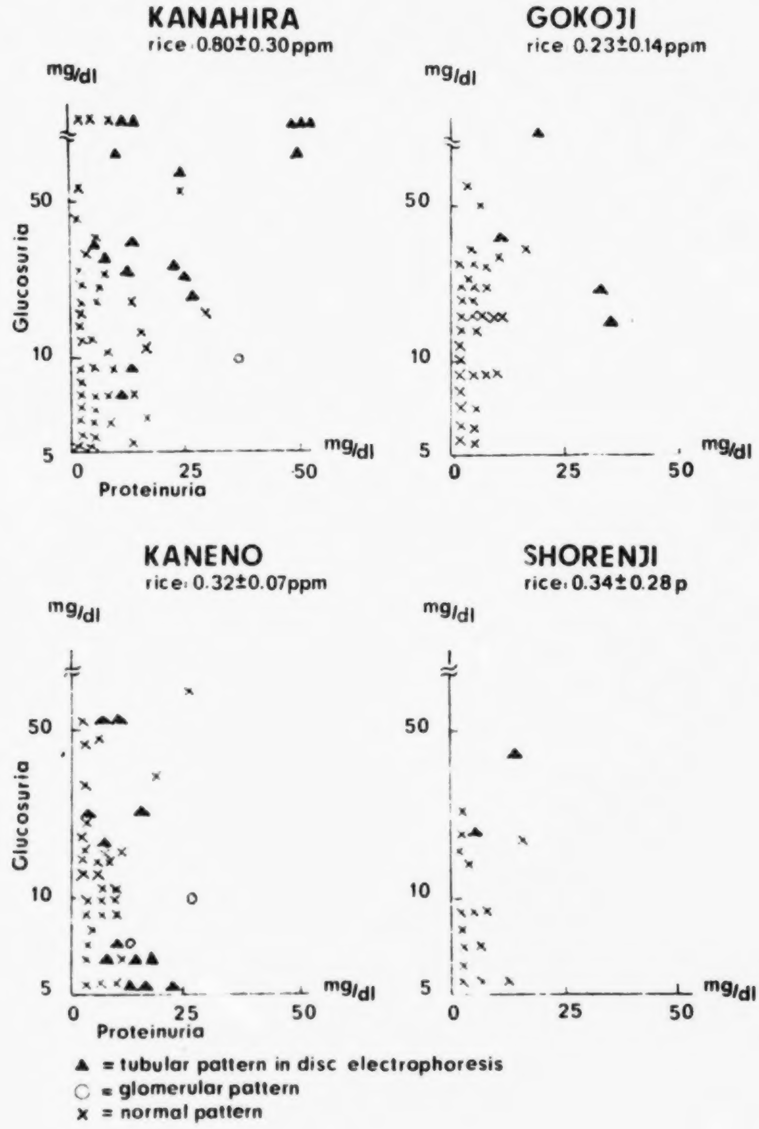


FIGURE 6:11 Results of urine analysis of men and women over 50 years of age in Kakehashi river basin. (From Ishizaki, 1972).

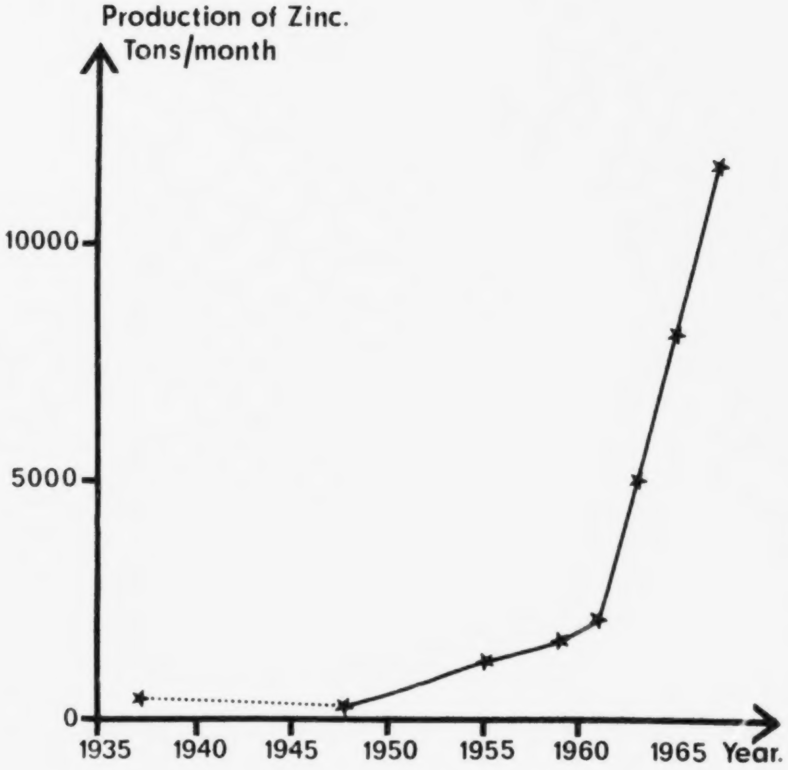


FIGURE 6:12 Changes in zinc production with time in the Annaka refinery. (From Mr. Fujimori, vice-president of the Annaka refinery, pers. comm.).

CHAPTER 7 THE CAUSE OF THE ITAI-ITAI DISEASE

In our previous review, Cadmium in the Environment (CITE) it was concluded that all available data strongly supported the conclusion that cadmium is a cause of the Itai-itai disease. It was stated, however, that it might well be that cadmium had acted upon a population particularly sensitive because of deficient consumption of certain essential food ingredients and vitamins, particularly calcium and vitamin D.

It is not the aim of this report to go into details about the Itai-itai disease. However, since CITE was published, a new hypothesis about its etiology has been introduced by some researchers (Takeuchi and Naito, 1972) in Japan. They argue that the Itai-itai disease might have been caused by an initial vitamin D deficiency and a subsequent vitamin D poisoning. The article in which this hypothesis is introduced is written in the form of a discussion and the hypothesis is not supported by any factual data.

The hypothesis implies that vitamin D deficiency should be much more pronounced in Fuchu than in other areas of Toyama Prefecture. There is no substantiation that this is the case. As was mentioned in CITE, the dietary conditions in Fuchu did not differ from those in neighboring areas, where the disease was not found. Still more inconsistent with the vitamin D hypothesis is the fact that when the Itai-itai disease did occur in Toyama areas outside Fuchu, it only occurred in areas irrigated by the Jintsu River. Moreover, the fact that in most cases it was necesssary to give very large doses of vitamin D over prolonged periods definitely does not speak in favor of vitamin D deficiency alone as the cause of osteomalacia; instead, it strongly favors that the osteomalacia was secondary to some renal damage.

According to Hagino, 1973, 30 Itai-itai patients were treated before 1948 and 1950 with 5-10 g cod liver oil (corresponding to about 1,000 I.u. vitamin D) per day for 3-6 months, but the bone symptoms did not recede. This dose should be sufficient for treatment of vitamin D deficient osteomalacia (Documenta Geigy, 1960, Merck Manual, 1966, and Arnstein, Frame and Frost, 1967). Hagino, 1957, and Nakagawa and Furumoto, 1957, report that large doses of Vitamin D are needed to reverse the bone symptoms. Hagino, 1973, gave 50,000-100,000 I.u./day in 1955-1957 and 100,000 I.u. per day during 1967-1969. When treatment was interrupted (Hagino, 1973) the bone symptoms returned. Nakagawa, 1960, reported on 30 patients, whose treatment started in 1955-1958. To out-patients, an average of 20,000 I.u vitamin D/day was given and to in-patients, 100,000 I.u./day. In many cases, the pain was relieved after a couple of months and the patients could resume their everyday lives. Murata, Nakagawa and Hirono, 1972, reported that 100,000-200,000 I.u./day had been used for years on 5 of these patients.

As mentioned, Takeuchi and Naito, 1972, also argued that treatment with large doses of vitamin D could have been the cause of kidney damage in Itai-itai disease. To this can be said first of all that cadmium-induced dose-related proteinuria is seen in many persons (also in men) other than Itai-itai patients (see section 6.3.1). There are no data that such a massive outbreak of proteinuria in men as well as in women could have been caused by excessive treatment with vitamin D. Furthermore, kidney damage was common in Itai-itai patients already in the 1950's, i.e. even in patients that had not received vitamin D treatment. Backing for this assertion can be found e.g. in the report of Nakagawa, 1960, describing 30 Itai-itai patients from the Shinbo district who were studied 1955-1958. All of the patients had proteinuria (Sulfosalicylic acid method) and some had glucosuria (Nylander method) as well. Vitamin D had not been taken by the patients before they were examined (Nakagawa, pers. comm.).

There are some other data related to postulations about kidney damage in the mid-1950's. Taga et al., 1956, reported bone symptoms among 10 patients. Two of these patients had died in 1955, and were autopsied by Kajikawa. He reported (Kajikawa et al., 1957) that these patients must have suffered severe kidney damage. They could not have been treated with high doses of vitamin D over long periods of time since vitamin D treatment was not started before 1955 (Hagino, 1973). Hagino, 1957, reported that proteinuria (82%) and glucosuria (32%) were common signs among Itai-itai patients.

Takeuchi and Naito, 1972, also emphasize that in spite of extensive health screenings in other cadmium polluted areas of Japan, no Itai-itai disease patients have been found. They present this as an argument against cadmium as an etiological agent. It is true that Itai-itai disease has been extremely rare in areas other than Fuchu. We know of only two recent suspected cases, both from the Ikuno area (one discovered during the work of the Study Group for the Differential Diagnosis of Itai-itai Disease and Cadmium Poisoning and one reported by Kumagawa et al., in press).

Whatever the mechanism behind these two cases may be, the fact that Itai-itai disease has not been acknowledged in areas other than Fuchu does not weaken the conclusion in CITE that cadmium was a cause of the disease. There is reason to believe that exposure to cadmium in the Fuchu area (section 6.3.1) has been extremely high. Values of above 2 ppm cadmium in rice were sometimes found and values between 1.0 and 1.9 were not uncommon. Moreover, as discussed in CITE, the exposure in the past was probably higher. The combination of very high, very long cadmium exposure and poor nutrition may explain why Itai-Itai disease has been seen only in Fuchu.

What has been mentioned above does not of course exclude the possibility that among the approximately 200 cases of recognized Itai-itai disease, sporadic cases might involve "bone and kidney disease unrelated to cadmium exposure".

As was pointed out in section 6.4, the epidemiological studies in Japan have had as a primary aim to detect possible cases of Itai-itai disease. It must be realized that with this approach it is not possible to make a proper evaluation of less severe manifestations of chronic cadmium poisoning.

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